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Cryptosporidiosis

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INTRODUCTION

Organisms of the genus *Cryptosporidium* are small coccidian parasites that infect the microvillous region of epithelial cells lining the digestive and respiratory organs of vertebrates (8, 73, 75, 101, 322). Recognized and named over 80 years ago (318–320), these small (2 to 6 μm, depending on stage of life cycle), obligate, intracellular protozoans remained until recently nothing more than a biomedical curiosity. Prior to 1980, infections with species of *Cryptosporidium* were considered rare in animals, and in humans they were thought to be the result of a little-known opportunistic pathogen of immune deficient individuals outside its normal host range. Beginning in 1982, our concept of these protozoan parasites changed to the consideration that they are

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important, widespread causes of diarrheal illness in humans and some domesticated animals. In immunocompetent persons, *Cryptosporidium parvum* may cause a short-term (3- to 20-day) diarrheal illness that resolves spontaneously. However, in the immunocompromised patient, cryptosporidiosis usually presents as a life-threatening, prolonged, choleralike illness. At the time of this writing, no effective therapy for cryptosporidiosis has been identified; thus, the finding of this parasite in the immunocompromised host, especially patients with AIDS, usually carries an ominous prognosis. Reports of respiratory tract (106, 195, 317) and biliary tree (249) infections demonstrate that the developmental stages of this protozoan are not always confined to the gastrointestinal tract and suggest that *C. parvum* may be an underreported cause of respiratory and biliary tract disease, especially in the immune deficient host.

Recent recognition of the importance of *Cryptosporidium* spp. as human and domesticated animal pathogens can be confirmed easily by the number of relevant publications that have appeared in the biomedical literature. Less than 30 papers addressing these parasites were published prior to 1980; however, at the time of this writing, more than 950 papers on *Cryptosporidium* spp. and cryptosporidiosis exist. Among the many recent papers are several reviews of the biology of *Cryptosporidium* spp. (67, 73, 101, 113). In this communication those aspects of most importance to the clinical microbiologist will be addressed.

HISTORY

Clarke (58), in 1895, may have been the first to observe a species of *Cryptosporidium* which he described as "swarm spores lying upon the gastric epithelium of mice." In retrospect, these small organisms were probably the motile merozoites of *C. muris*, the type species named and described approximately 12 years later by the well-known American parasitologist, E. E. Tyzzer (318). This small coccidian, infecting the gastric epithelium of laboratory mice (*Mus musculus*, Japanese waltzing mice, and English mice) used in Tyzzer's research program, was placed in a new genus (*Cryptosporidium* = hidden sporocysts) because, unlike the previously known coccidia, the oocyst of this parasite did not have sporocysts surrounding the sporozoites. Approximately 3 years later, Tyzzer (319) described many of the life cycle stages of *C. muris*, and in 1912 he (320) described much of the morphology and life cycle of a second species, *C. parvum*, found in the small intestine of laboratory mice. Approximately 17 years later, Tyzzer (321) described and illustrated the developmental stages of a species of *Cryptosporidium* in the cecal epithelium of chickens. Relatively little detail was included in this description because he thought it was *C. parvum*, the species he had described previously.

During the ensuing half-century following Tyzzer's original reports of *C. muris* and *C. parvum*, these protozoans were not regarded as economically or medically important and, therefore, received little attention for biomedical researchers. Studies conducted from 1961 to 1986 that relied primarily on structural features of oocysts resulted in the naming of approximately 19 additional species of *Cryptosporidium* from fishes, reptiles, birds, and mammals (73, 101, 182). Only a few of these named species, including the two originally described by Tyzzer, are now considered valid (see below).

The 1955 report of Slavin was the first to associate cryptosporidiosis with morbidity and mortality. He described a severe diarrhea and some deaths in 10- to 14-day-old turkey poults and attributed the illness to a new species of *Cryptosporidium*, *C. meleagridis* (287). Interest in *Cryptosporidium* (*C. parvum*) by the veterinary medical profession was stimulated in 1971 when this protozoan was first reported to be associated with bovine diarrhea (242). Since this time, numerous case reports from many different animals are now present in the literature and one species, *C. parvum*, is recognized as an important cause of neonatal diarrhea in calves and lambs (8, 73, 322). Another species, *C. baileyi*, is now recognized as an important cause of respiratory disease in poultry (34, 84, 85).

The first cases of human cryptosporidiosis were reported in 1976 (216, 236), and subsequent reports were rare until it was recognized that *Cryptosporidium* (now believed to be *C. parvum*) may produce a short-term diarrheal illness in immunocompetent persons and a prolonged, life-threatening, choleralike illness in immune deficient patients, especially those with AIDS (67, 73, 75, 82, 101). Additional details of the historical events outlined above can be found in review papers published between 1983 and 1989 (8, 67, 73, 75, 101, 113, 230, 322).

TABLE 1. Taxonomic classification of *Cryptosporidium*

Classification	Name	Biological characteristics
Phylum	Apicomplexa	Invasive forms have apical complex with polar rings, rhoptries, micronemes, conoid, and subpellicular microtubules
Class	Sporozoasida	Locomotion of invasive forms by body flexion gliding, or undulation
Subclass	Coccidiasina	Life cycle with merogony, gametogony, and sporogony
Order	Eucoccidiorida	Merogony present; found in vertebrate hosts
Suborder	Eimeriorina	Male and female gametes develop independently
Family	Cryptosporidiidae	Homoxenous (one host life cycle), with developmental stages just under the membrane of the host cell; oocyst without sporocysts and with four sporozoites; microgametes without flagella

CLASSIFICATION

The taxonomic classification of small intracellular protozoans assigned to the genus *Cryptosporidium* is presented in Table 1. Species of *Plasmodium* causing malaria in humans are in the same order (Eucoccidiorida) but in a different suborder (Haemospororina) than species of *Cryptosporidium*. More closely related to *Cryptosporidium* spp. are the other true coccidia (suborder Eimeriorina), *Isospora belli*, *Sarcocystis* spp., and *Toxoplasma gondii*, which infect human beings, and *Eimeria* spp., which infect other mammals and birds. Most species of *Cryptosporidium* named in the biomedical literature following Tyzzer's creation of the genus were done so with the assumption that these coccidia were as host specific as the closely related (taxonomically) species of *Eimeria* infecting mammals and birds. However, cross-transmission studies conducted in the early 1980s demonstrated little or no host specificity for "species" of

Cryptosporidium isolated from mammals. The lack of host specificity exhibited by mammalian isolates prompted Tzipori et al. (325) to consider *Cryptosporidium* as a single-species genus. A more realistic approach was presented by Levine (182), who consolidated the 21 named parasites into four species, one each for those infecting fishes (*C. nasorum*), reptiles (*C. crotali*), birds (*C. meleagridis*), and mammals (*C. muris*). Information available at the time of this writing indicates that this consolidation is not entirely correct. *C. crotali* is now considered to be a species of *Sarcocystis*, a genus of coccidian parasites found commonly in snakes. At least two valid species, *C. baileyi* and *C. meleagridis*, infect birds (85), and also at least two valid species infect mammals (*C. parvum* infecting the small intestine and *C. muris* infecting the stomach). On the basis of oocyst morphology, *C. parvum*, not *C. muris*, is associated with all well-documented cases of cryptosporidiosis in mammals (337). Ultrastructural studies also support the view that *C. parvum* and *C. muris* are distinct species (81, 336). Thus, at the time of this writing, the species with oocysts measuring 4 to 5 μm that produces clinical illness in humans and other mammals should be referred to as *C. parvum*, or *Cryptosporidium* sp. if there are not enough morphologic, life cycle, and/or host specificity data to relate it to Tyzzer's original description. We have adopted this conservative approach realizing that careful studies of proposed differences in host specificity, sites of infection, and pathogenicity among mammalian isolates (73, 101, 322) may result in the validation of additional species. In light of the present uncertainties in the taxonomy of *Cryptosporidium* spp., it is preferable to designate a particular parasite obtained from a mammalian host as an isolate rather than a strain. Recently, reverse transcription of total cellular RNA was used to obtain a partial sequence of the small-subunit rRNA of *Cryptosporidium*. The results did not show an especially close relationship between *Cryptosporidium* and other members of the phylum Apicomplexa (149). With the use of newer, more sophisticated techniques, the classification of *Cryptosporidium* may undergo additional changes in the future.

LIFE CYCLE

Studies of different isolates (calf and human) of *C. parvum* in suckling mice (81) revealed that the life cycle of this parasite (Fig. 1 and 2) is similar to that of other true coccidia (e.g., *Eimeria* and *Isospora* spp.) infecting mammals in that it can be divided into six major developmental events: excystation, the release of infective sporozoites; merogony, the asexual multiplication within host cells; gametogony, the formation of micro- and macrogametes; fertilization, the union of micro- and macrogametes; oocyst wall formation, to produce an environmentally resistant stage that transmits infection from one host to another; and sporogony, the formation of infective sporozoites within the oocyst wall. The life cycle of human and calf isolates of *C. parvum* differs somewhat from that of other monoxenous (one host in life cycle) coccidia such as *Eimeria* and *Isospora* spp., parasites usually presented as the "typical" coccidia. Each intracellular stage of *C. parvum* resides within a parasitophorous vacuole confined to the microvillous region of the host cell, whereas comparable stages of *Eimeria* or *Isospora* spp. occupy parasitophorous vacuoles deep (perinuclear) within the host cells. Oocysts of *C. parvum* undergo sporogony while they are within the host cells and are infective when released in the feces, whereas oocysts of *Eimeria* or *Isospora* spp. do not sporulate until they are passed from the host and exposed to oxygen and temperatures below 37°C. Studies with experimentally infected mice have also shown that approximately 20% of the oocysts of *C. parvum* within host enterocytes do not form a thick, two-layered, environmentally resistant oocyst wall. The four sporozoites of this autoinfective stage are surrounded only by a single unit membrane. Soon after being released from a host cell, the membrane surrounding the four sporozoites ruptures and these invasive forms penetrate into the microvillous region of other enterocytes and reinitiate the life cycle (81). Approximately 80% of the oocysts of *C. parvum* found in enterocytes of suckling mice were similar to those of *Eimeria* and *Isospora* spp. in that they developed thick, environmentally resistant oocyst walls and were passed in the feces. Thick-walled oocysts are the life cycle forms that transmit the infection from one host to another. The presence of autoinfective, thin-walled oocysts and type I meronts that can recycle are believed to be the life cycle features of *C. parvum* responsible for the development of severe infections in hosts exposed to only a small number of thick-walled oocysts and for persistent, life-threatening disease in immune deficient persons who are not exposed repeatedly to these environmentally resistant forms. Light microscopic and ultrastructural features of some of the developmental stages of *Cryptosporidium* in enterocytes of the experimentally infected host are shown in Fig. 2 to 5. Additional details of the ultrastructure of *Cryptosporidium* spp. can be found in several publications (81, 117, 256, 336).

Studies (85) of *C. baileyi* in experimentally infected chickens have revealed that this species has a life cycle similar to that described above for *C. parvum* in suckling mice. The major difference in the life cycle of these two species is that *C. baileyi* has three distinct types of meronts rather than the two types found in *C. parvum*.

CULTIVATION

Following the development of techniques to purify oocysts from calf feces, sterilize the purified preparation, and obtain viable sporozoites, the growth of *C. parvum* in chicken embryos was successful (79). Both human and calf isolates completed their entire life cycles (from sporozoite to sporulated oocyst) in endoderm cells of the chorioallantoic membrane (CAM) of chicken embryos. The morphology, time of appearance, and sequence of development of *C. parvum* in the CAM on days 1 to 8 after sporozoite inoculation were similar to those reported for the parasite growing in the ileum of experimentally infected mice inoculated orally with the same species (81). Subsequent to reporting success in culturing *C. parvum* in chicken embryos, it was discovered that the source of embryos is very important. Virtually all embryos from one supplier supported development of large numbers of parasites, whereas only 10 to 20% of embryos from two other sources supported parasite growth. The reason(s) for marked differences in susceptibility of chicken embryos obtained from different sources remains unresolved. With access to the proper source of embryos, the in ovo cultivation system can be manipulated for use in screening candidate therapeutic agents.

Use of the in ovo system to obtain large numbers of *C. parvum* for studies of parasite metabolism and immunology has been disappointing because of limited parasite growth and because most oocysts developing in the CAM are not released from the host cells into the allantoic fluid. Also, separation of developmental stages of the parasite from host

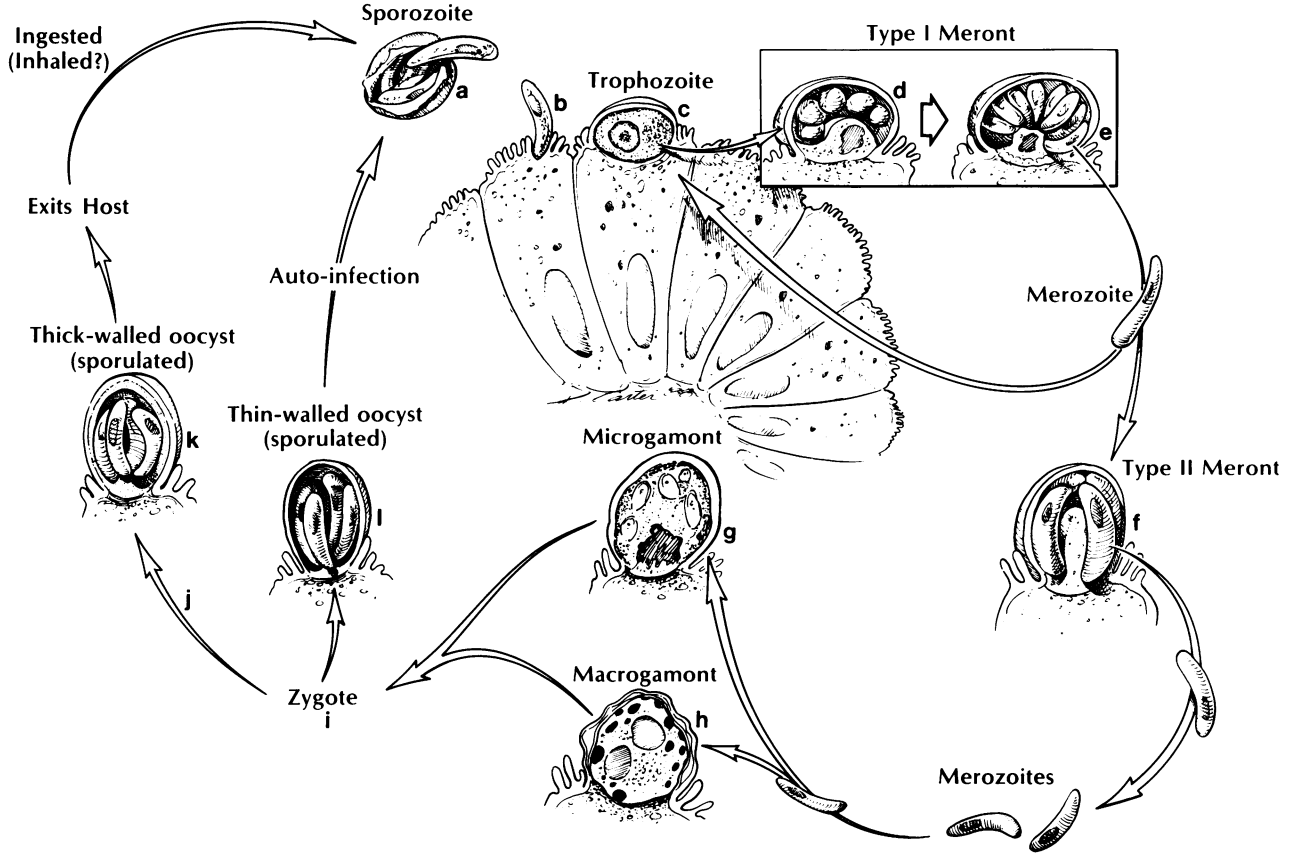

FIG. 1. Diagrammatic representation of the proposed life cycle of *C. parvum* as it occurs in the mucosal epithelium of an infected mammalian host. Living developmental stages of *C. parvum* corresponding to those labeled a through l in this life cycle diagram are shown in Nomarski interference contrast photomicrographs contained in Fig. 2. After excysting from oocysts in the lumen of the intestine (a), sporozoites (b) penetrate into host cells and develop into trophozoites (= uninucleate meronts) (c) within parasitophorous vacuoles confined to the microvillous region of the mucosal epithelium. Trophozoites (uninucleate meronts) (c) undergo asexual division (merogony) (d and e) to form merozoites. After being released from type I meronts, the invasive merozoites enter adjacent host cells to form additional type I meronts (recycling of type I meronts) or to form type II meronts (f). Type II meronts do not recycle but enter host cells to form the sexual stages, microgamonts (g) and macrogamonts (h). Most (approximately 80%) of the zygotes (i) formed after fertilization of the microgamont by the microgametes (released from microgamont) develop into environmentally resistant, thick-walled oocysts (j) that undergo sporogony to form sporulated oocysts (k) containing four sporozoites. Sporulated oocysts released in feces are the environmentally resistant life cycle forms that transmit the infection from one host to another. A smaller percentage of zygotes (approximately 20%) do not form a thick, two-layered oocyst wall; they only have a unit membrane surrounding the four sporozoites. These thin-walled oocysts (l) represent autoinfective life cycle forms that can maintain the parasite in the host without repeated oral exposure to the thick-walled oocysts present in the environment. The life cycle of *C. baileyi*, infecting chickens, differs from the one shown in that this parasite has an additional type (type III) of meront derived from type II merozoites. Drawing by Kip Carter, University of Georgia. Reprinted from *Coccidiosis of Man and Domestic Animals*, p. 155–185, with permission of the authors (W. L. Current and B. L. Blagburn) and CRC Press, Inc. (77a).

tissues is difficult. Growing *C. parvum* in cultured cells can also be disappointing when the goal is to obtain large numbers of organisms free of the microbial contaminants normally found in the host gut.

With some refining of the oocyst purification techniques used in the embryo culture studies described above, complete development of *C. parvum* in several cell types (human fetal lung, porcine kidney, and primary chicken kidney) was achieved. However, the number of oocysts produced in the cell culture was less than that produced in the intestines of suckling mice or in the CAM of chicken embryos (78). This reduced proliferation in cell culture was attributed to the absence of the autoinfective oocysts that develop in the mouse intestine and in the CAM of chicken embryos. Prolonged culture-to-culture passage of the parasite and the production of large numbers of parasites in vitro await elucidation of the right combination of growth conditions and host cells that will stimulate and support the autoinfective cycle occurring in the mammalian gut and in the chicken embryo. Recently, Datry et al. (87) reported that CACO2 cells, a human colon carcinoma cell line that expresses some characteristics of enterocytes in culture, also supports development of *C. parvum*. They reported that enough oocysts were obtained from the culture fluid of the infected CACO2 cells to initiate infections in another cell culture. The existence of autoinfective stages in this culture system has not been determined, and the number of serial passages of the parasite has not been verified. Monitoring numbers of developmental stages of *C. parvum* in mouse L929 cells has been reported as a useful in vitro model to evaluate drugs for

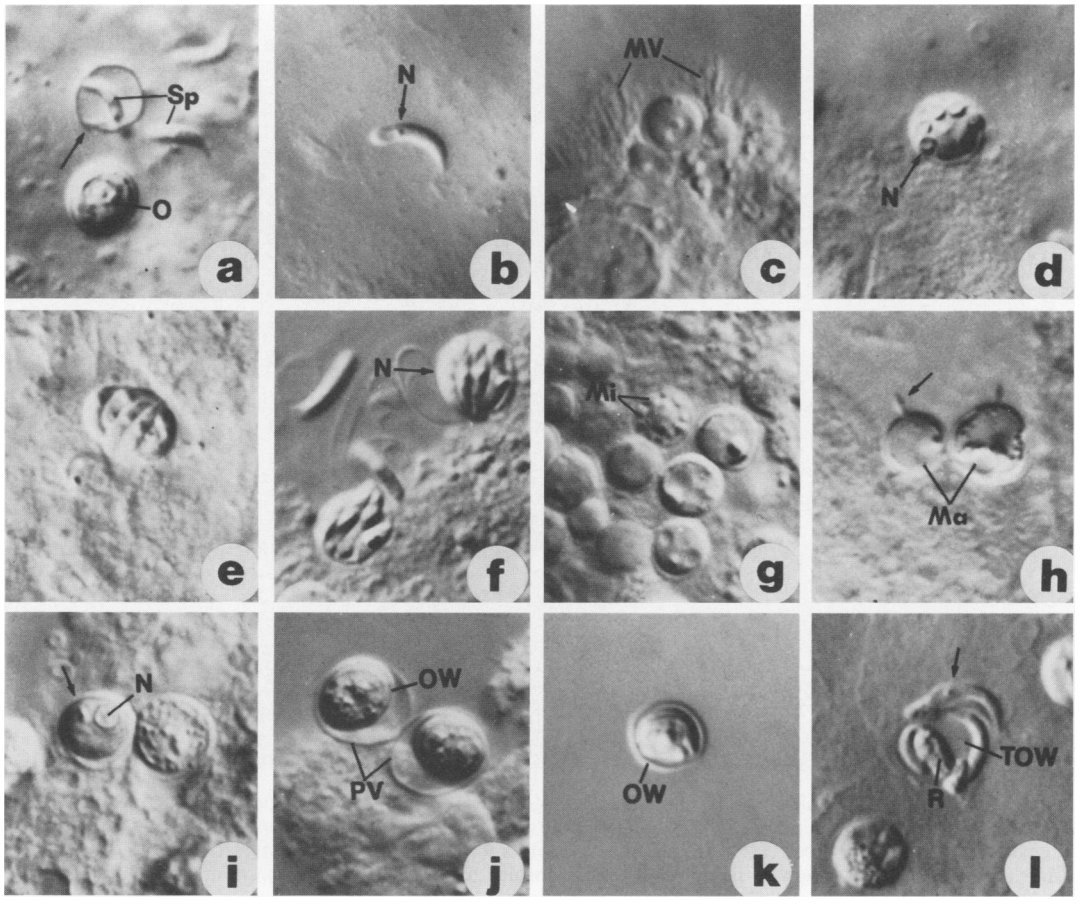

FIG. 2. Nomarski interference-contrast photomicrographs of developmental stages of *C. parvum* in mucosal scrapings obtained from the small intestines of experimentally infected suckling mice. (a) Sporozoites (Sp) free and excysting from the opening (arrow) in an oocyst, and an intact oocyst (O). (b) Free sporozoite showing the posterior location of the nucleus (N). (c) Trophozoite (uninucleate meront) surrounded by hypertrophied microvilli (MV). (d) Immature type I meront with peripherally located nuclei (N), six of which are in focus. (e) Mature type I meront containing six or eight merozoites. (f) Mature type II schizont (meront) showing the four merozoites arranged like the segments of an orange. Nuclei (N) of all four merozoites are aligned in the center of the meront. (g) Microgamont with microgametes (Mi) budding from the surface of the residuum. (h) Two macrogamonts (Ma) each with an attached microgamont (arrow points to one that is in focus). (i) Two macrogamonts (Ma), one of which contains a microgamont (arrow). (j) Two oocysts with thick walls (OW), both within parasitophorous vacuoles (PV). (k) Intact thick-walled oocysts that will pass unaltered in the feces. (l) An autoinfective, thin-walled oocyst that has ruptured under coverslip pressure, releasing the four sporozoites from the thin oocyst wall or membrane (TOW). Note the granular oocyst residuum (R) and the posterior location of the sporozoite nuclei (arrow). Adapted from reference 81. Reprinted with permission of the publisher.

potential anti-*Cryptosporidium* activity (210). To date, attempts in several laboratories to grow *C. baileyi* in cell culture have been unsuccessful (184).

EPIDEMIOLOGY

Transmission by Environmentally Resistant Oocysts

Studies of experimental infections in laboratory and farm animals clearly demonstrate that *C. parvum* is transmitted by environmentally resistant oocysts that are fully sporulated and infective at the time they are passed in feces (7, 73, 82). As long as the thick two-layered wall remains intact, *Cryptosporidium* oocysts are very resistant to most common disinfectants, and they can survive for months when kept cold and moist. One study (308) designed to evaluate the efficacy of commercial disinfectants demonstrated that exposure to ammonia (50% or higher) and formalin (10% or higher) for 30 min can kill *Cryptosporidium* oocysts. When these disinfectants and others used routinely in hospitals and clinical laboratories were evaluated at the lower concentrations recommended by the manufacturers, none were effective against *Cryptosporidium* oocysts. Freeze-drying and exposure (30 min) to temperatures above +60°C and below −20°C have also been reported to kill *Cryptosporidium* oocysts (6, 322). Most *C. parvum* oocysts stored at 4°C in 2.5% (wt/vol) aqueous potassium dichromate solution remain viable for 3 to 4 months, and some may remain infective for cell cultures and suckling mice for >1 year (73).

The recent documentation of waterborne transmission of *C. parvum* and the demonstration of oocysts in potable water samples (see below) are of concern to the water industry and have prompted several studies to evaluate disinfectants commonly used for water treatment. The earliest data suggesting that routine chlorination of drinking water has little or no effect on oocyst viability stemmed from procedures used routinely in several laboratories to sterilize *Cryptosporidium* oocysts prior to obtaining viable sporozo-

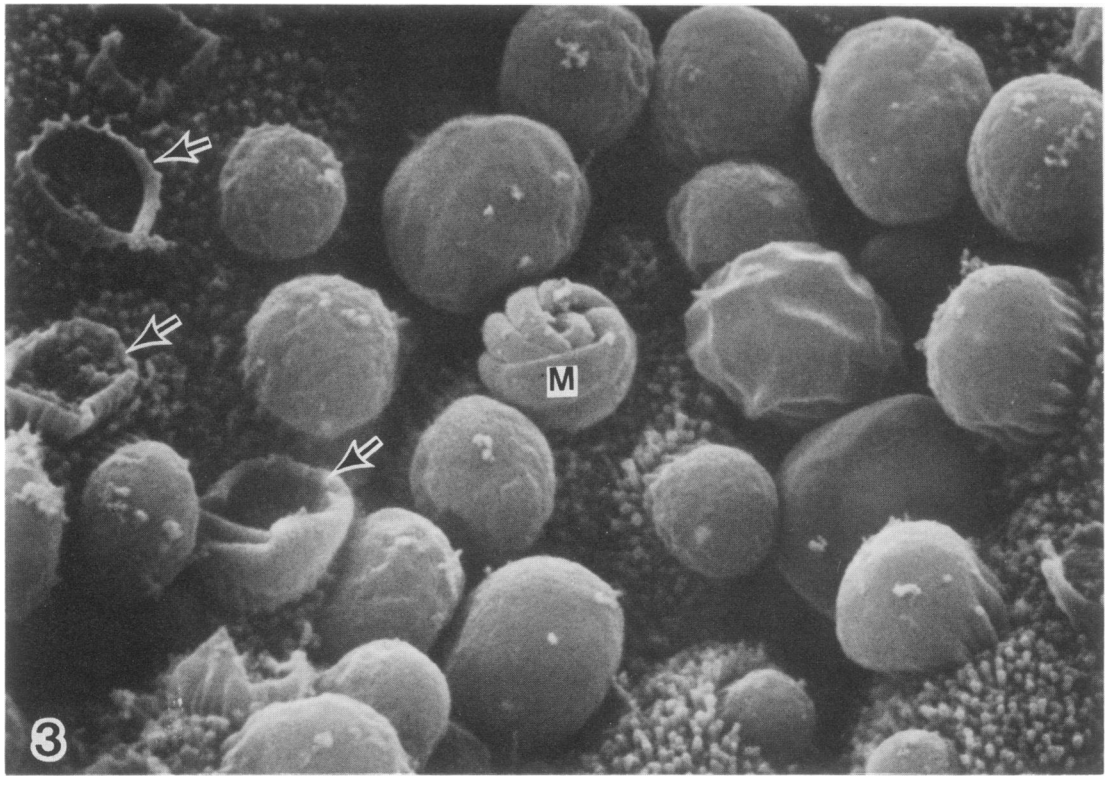

FIG. 3. Scanning electron micrograph showing numerous developmental stages of *Cryptosporidium* in the microvillous region of the intestinal mucosa. Each parasite is contained within a parasitophorous vacuole that bulges out from the microvillous region of the enterocyte. Some merozoites of a mature type I meront (M) are exposed as a result of a portion of the parasitophorous vacuole membrane being removed during processing. Arrows point to craters in the mucosal surface formed by empty vacuoles that remain after the parasites are released.

ites by in vitro excystation. This procedure involves incubating oocysts in 10 to 50% commercial bleach (0.5 to 2.5% sodium hypochlorite) for 10 to 15 min in an ice bath. Skeptics argued that these data are difficult to interpret because of the low incubation temperatures, the short incubation times, and the possible organic (fecal) contamination that can cause a high disinfection demand. The argument for high disinfection demand is not valid because oocysts tested in our laboratory (and other laboratories) were highly purified. More recent studies with disinfectants commonly used to treat water have been performed with purified oocysts (a demand-free situation) and different incubation times and temperatures. In one carefully controlled study, oocyst viability, as determined by prevention of excystation or infectivity, was abolished following exposure to 80 ppm of chlorine at 25°C, pH 7.0, for 2 h. With these data, the $C \cdot t'$ (concentration $\times$ time required for killing) value for *C. parvum* oocysts was 9,600 compared with a $C \cdot t'$ of <15 for *Giardia* cysts (170). In the same study, ozone (another popular method for water treatment) was shown to eliminate infectivity of *C. parvum* oocysts when kept at a concentration of 1 ppm for 10 min. Under normal operating conditions, water utilities attempt to maintain a residual activity of 1.0 ppm of chlorine and 0.4 ppm of ozone; however, the latter is extremely unstable, and its activity cannot be effectively maintained. Results from this study also indicate that *C. parvum* oocysts are 30 times more resistant to ozone and 14 times more resistant to chlorine dioxide than are *Giardia lamblia* cysts exposed under the same conditions (170). Thus, it appears that routine chlorination or ozonation used for most waterborne organisms will have little effect on the viability of *C. parvum* oocysts.

Sources of Human Infection

Data published from several laboratories during the early 1980s demonstrated that calves are a source of human infection (7, 82, 257). Companion animals such as rodents, puppies, and kittens may also serve as reservoir hosts (82). These findings, in conjunction with reports of more than 40 mammals that harbor the parasite (75) and the realization that *C. parvum* readily crosses host species barriers, led to the concept that most human infections are a result of zoonotic transmission. This view is probably correct for persons living and working in environments where exposure to fecal contamination (especially waterborne) from potential reservoir hosts is likely. However, zoonotic transmission cannot explain the large number of infections reported from persons living and working in urban areas where exposure to animal feces is minimal. Present evidence indicates that person-to-person transmission of cryptosporidiosis is common (3, 30, 45, 94, 217, 237, 341). In 1983, an accidental laboratory infection demonstrated that a human isolate of *C. parvum* could be transmitted from one person to another (33). Since that time, outbreaks of cryptosporidiosis among children in day-care centers have been reported (2, 3, 56, 62, 93, 131, 172, 217, 237, 254, 292, 301), hospital-acquired infections have been investigated (28, 167, 203, 204), a number of waterborne outbreaks have been documented (86, 107, 129, 271, 290, 291), and this protozoan is now recog-

nized as a cause of traveler's diarrhea (16, 69, 95, 103, 151, 152, 282, 296, 304, 310). There is also concern that some food-borne organisms, such as parasitic protozoa (possibly including *Cryptosporidium*), which serve as hosts for unique bacterial and viral symbionts might also become infected with mammalian viruses, thus transmitting multiple infections (31, 146).

Waterborne Transmission

Cryptosporidiosis has recently joined the ranks of diseases transmitted by water. As mentioned above, a number of waterborne outbreaks have been documented (86, 107, 129, 271, 290, 291). The first documented waterborne outbreak of cryptosporidiosis occurred in San Antonio, Tex., and was linked to sewage leakage into well water (86). Water from this well was chlorinated but not filtered. During the summer of 1986, drinking water from a common reservoir was considered to be the only epidemiological source link to an outbreak of cryptosporidiosis among persons in Sheffield, England (271). Similar consumption of untreated surface water appeared to be the predominant risk factor associated with cryptosporidiosis among 78 laboratory-confirmed cases of cryptosporidiosis in New Mexico during the summer of 1986 (107). In January and February 1987, cryptosporidiosis was associated with an estimated 13,000 cases of gastroenteritis among residents of Caroll County, Georgia (129). *Cryptosporidium* oocysts were identified in the stools of 39% of the persons examined during the outbreak, and a randomized telephone survey suggested attack rates of 54% within the city of Carollton and 40% overall for the county. The only significant risk factor associated with illness was exposure to the public water supply which was filtered and chlorinated, and, according to records kept during the outbreak, the treatment facility was operating within established Environmental Protection Agency (EPA) guidelines. In 1988 and 1989, two additional *Cryptosporidium*-related waterborne outbreaks were reported in Ayshire, Scotland, and Oxfordshire-Swindon, England (290, 291).

The increase in reported waterborne disease outbreaks associated with *Cryptosporidium* spp. can be attributed in part to improvements in techniques to provide positive identification of the causative agent. After the first waterborne outbreak in San Antonio was investigated, we (83) demonstrated that some of the oocysts of *C. parvum* added to water samples can be recovered by high-volume filters designed to trap cysts of the enteric protozoan, *G. lamblia*. Application and further refining of similar recovery techniques, in conjunction with immunofluorescent detection methods, have resulted in the demonstration of *Cryptosporidium* oocysts in surface and drinking waters and in sewage effluent samples obtained from many different geographic regions of the United States and from several other countries (129, 144, 197, 229, 239, 269, 291, 304, 305). Wastewater in the form of raw sewage and runoff from dairies and grazing lands has been identified as a likely source of oocysts that contaminate drinking and recreational water. The importance of agricultural sources of oocyst contamination should not be taken lightly since infected calves and lambs can pass up to 10^{10} oocysts per day for up to 14 days (37). Thus, large numbers of oocysts can enter the surface water system following a hard rain on a pasture containing infected animals. The studies just reviewed, as well as the prevalence data discussed below, demonstrate that *C. parvum* is ubiquitous in the environment and that it is likely to be present as a waterborne pathogen, especially where standards of sanitation and water treatment technology are low.

Prevalence

Stool diagnosis. Human infections with *Cryptosporidium* (*C. parvum*) have been reported on six continents (Table 2). Most prevalence data contained in published surveys result from standard stool examination techniques to detect *C. parvum* oocysts. These data are quite variable even from one geographic location. Direct comparison of the results is often difficult because study populations may not be comparable and because different stool sampling and oocyst detection procedures were used. Aside from outbreak situations, most specimens included in surveys from developed countries are from adults or children whose fecal samples have been submitted to a specific diagnostic laboratory because of a gastrointestinal complaint. A number of field surveys have been conducted in developing countries. In spite of these difficulties, a data base is being compiled from which a limited understanding of the geographic distribution and prevalence of human cryptosporidiosis is beginning to emerge.

A review (101) of 36 large-scale surveys of selected populations, such as children and adults seeking medical attention for diarrhea and other gastrointestinal symptoms, demonstrates that *Cryptosporidium* sp. is associated with diarrheal illness in most areas of the world and that the prevalence of cryptosporidiosis is highest in poorly developed regions. For example, prevalence rates reported in surveys from Europe (1 to 2%) and North America (0.6 to 4.3%) are lower than those reported in surveys from Asia, Australia, Africa, and Central and South America (3 to 20%). In most of the surveys reviewed by Fayer and Ungar (101), *Cryptosporidium* sp. was the most common parasite found and, in several, this protozoan was considered to be the most significant of all known enteropathogens causing diarrheal illness. Other findings common to many of the surveys were that there was usually a significantly higher prevalence in children than in adults, prevalence was highest in children less than 2 years of age, and infections were often seasonal, with a higher prevalence during warmer, wetter months. Another interesting finding from the standpoint of infection control was that a small number of oocysts may be present in feces for up to 2 weeks following resolution of diarrhea.

Several additional reviews (67, 80, 113, 232, 324) of the published reports of cryptosporidiosis in persons residing in industrialized and developing countries support the overall conclusions presented above and provide a more global view of the prevalence of human infection. Crawford and Vermund (67) compared the worldwide occurrence of *Cryptosporidium* infection compiled by Navin (230) from studies prior to 1985 with that obtained from studies published from 1985 to 1988. Data compiled from the pre- and post-1985 studies were similar. Studies prior to 1985 suggested that the overall prevalence of *Cryptosporidium* infection in individuals with diarrhea was 2.5% (19 of 7,779) for persons living in industrialized countries and 7.2% (82 of 1,135) for persons residing in developing countries (230). The more recent studies summarized by Crawford and Vermund suggested that the infection rate for individuals with diarrheal illness was 2.2% (285 of 11,716) for individuals in industrialized countries and 8.5% (532 of 6,295) for individuals in developing countries.

A summary of more than 100 geographically based surveys (published between 1983 and 1990) for the presence of

TABLE 2. Summary of reports (1983–June 1990) of *Cryptosporidium* sp. oocysts in stool specimens from different geographic study populations

Yr	Location	No.[a]		% Positive	Diagnostic method[b]	Comment(s)[c]	Reference
		Patients	Specimens				
1983	Australia	884	884	4.1	Giemsa	All symp; adults (low), children (high)	328
	Finland	154	1,422	9.1	F-E concn, Ziehl-Neelsen (cold); Giemsa	Adults only	151
	United Kingdom	500	500	1.4	Ziehl-Neelsen (hot)	All symp; adults (low), children (high)	54
1984	Costa Rica	278	278	4.3	Giemsa	All symp	206
	Denmark	800	1,200	2.0	F-E concn, mod Ziehl-Neelsen	All symp	141
	Peru	111	111	8.1			277
	Rwanda	293	293	7.8	Safranin 1%, more sensitive than mod Ziehl-Neelsen	All symp; adults (3%), children (10.4%); assoc with malnutrition	39
	Rwanda	72	72	11.1	F-E concn, safranin	All children, measles assoc	89
	United Kingdom	867	867	5.0	Ziehl-Neelsen (cold)	All symp	143
	United Kingdom	1,967	2,369	1.4	Sucrose flotation; Ziehl-Neelsen (cold)	Children only; all symp	127
1985	Canada	1,621	2,252	1.2	Auramine; pos confirmed by Kinyoun acid fast	18/19 gastroenteritis; more severe diarrhea in infants and children	255a
	Brazil	117	117	8.0	Auramine-rhodamine; Kinyoun acid fast	All symp	343
	United States (Massachusetts)	1,703	2,821	2.8	Kinyoun acid fast	43/47 immunocompetent; <4 yr and 30–39 yr (high); some association with giardiasis	352
	Finland	4,545	5,730	2.6	F-E concn, mod Ziehl-Neelsen	Most patients had recent travel; no cases <5 yr; 6.2% young adults	152
	Spain	NR	339	0.9	Ziehl-Neelsen (cold)		187
	Venezuela	120	120	10.8	Giemsa and/or mod Ziehl-Neelsen	Children <2 yr; symp	245
	United Kingdom	213	213	3.2	Mod Ziehl-Neelsen (cold)	0.9% of 112 controls pos; children only/symp	145
	Mexico	57	57	32.0			297
	Bangladesh	578	578	4.3	Ziehl-Neelsen (cold)	All symp	279
	France	190	200	2.1	Sugar flotation, wet mounts	All children; symp	13
	India	682	682	13.1	Concn, S-MB	All acute diarrhea; 9.8% pos in 418 controls; all seasons	207
	Canada	7,300	7,300	0.63	Ziehl-Neelsen (cold)	All symp; cause of summer diarrhea	224
1986	Ghana	474	474	12.9	Mod Ziehl-Neelsen	More common 2–12-mo age group; important childhood disease here	1
	United States (Oregon)	1,710	1,710	0.35	Auramine O; mod Ziehl-Neelsen	Not associated with giardiasis; however, 12.5% positives with *Giardia*	286
	Liberia	374	374	8.4	F-E concn, mod Ziehl-Neelsen	6–59-mo age group; 8.4% pos with diarrhea; 5.9% pos asymp; <2.5 yr (high); bottle fed (high); breast fed (low)	140
	United States (Pennsylvania)	53	53	43.0	Rapid DMSO-mod acid fast	Outbreak/day-care center; 14% pos household contacts; 65% pos symp; 11% pos asymp; suggested person-to-person transmission	3

Continued on following page

TABLE 2—*Continued*

Yr	Location	No.[a]		% Positive	Diagnostic method[b]	Comment(s)[c]	Reference
		Patients	Specimens				
	United Kingdom	4,028	4,028	1.6	S-MB	All symp; <12 mo (high); July + Sept down; Feb + Apr up; recommendation: screen symp children	27
	New Zealand	1,273	1,669	4.2	Mod Ziehl-Neelsen (cold)	7.2% pos in ages 1–15; 40% in spring and early summer; recommendation: screen all with diarrhea	50
	Czechoslovakia	1	1	100.0	Sucrose flotation	First reported case (4 yr old)	148
	Finland	5	5	100.0	F-E concn, acid fast	Veterinary student outbreak; 1–13-day symp; all diarrhea	252
	Sudan	83	83	6.1	S-MB	All children; all symp and dehydrated; 37 asymp, all neg	267
	Canada	74	74	100.0	F-E concn; mod Kinyoun (cold)	All symp; 35/74 had been to Mexico; diarrhea 1–2 weeks; diarrhea 6 mo in compromised patients	95
	South Africa	259	259	11.9	Mod Ziehl-Neelsen	All children (symp); all hospitalized; all pos <2 yr; 103 asymp (all neg); only organism in 9.2% of children <2 yr old; 22.6% fatality	338
	Canada	3,656	3,656	1.0		All symp; <5 yr (high); >5 h (low); common late summer and fall; no established epidemiologic assoc with infected cattle	201
	Germany	142	142	14.0	Carbolfuchsin	All children (symp); <2 yr (diarrhea 19.9 days); >2 yr (diarrhea, 4.1 days); *Cryptosporidium* should be considered as cause of diarrhea in young children	130
	United States (Michigan)	42	42	64.0	DMSO/mod acid fast	Outbreak day-care center; symp 1 day–4 wk; oocyst excretion up to 48 days; both symp and asymp; recommend screens for children with diarrhea (especially day-care centers)	62
	Finland	68	136	100.0	Ziehl-Neelsen	All symp; asymp still passing oocysts 1–15 days (6.9-day avg); discussion of asymp shedders	150
1987	United Kingdom	NR	2,197	0.5	Ziehl-Neelsen	Hospital-based population; recommendation: screen only immunosuppressed and those with persistent diarrhea	203
	Haiti	824	824	16.7	Mod Ziehl-Neelsen	All <2 yr; all symp; <6 mo (low); >6 mo (high)	243
	Ireland	935	1,246	4.3	Auramine/carbolfuchsin; mod acid fast	All children; 3 wk–12 yr; all acute diarrhea 7–15 days; no travel history; Feb + June (higher) 23/41 farming or rural background; recent outbreaks of diarrhea in cattle	66
	United States (Florida)	102	102	31.4	Unconcn stool; mod acid fast	Day-care center outbreak; 12–35 mo; asymp oocyst shedding reported; 33% pos (children); 22% pos (staff); 101/102 diarrhea	301
	Germany	1,600	1,600	1.9	MIF concn; stain?	Evenly divided children, adults; all symp; oocyst excretion average: 14 days	298
	Nigeria	NR	479	2.3		Children (high, 5.3% of 150); rainy season higher; 6/11 *Cryptosporidium* only pathogen	260

Continued on following page

TABLE 2—*Continued*

Yr	Location	No.[a]		% Positive	Diagnostic method[b]	Comment(s)[c]	Reference
		Patients	Specimens				
	West Indies	NR	513	4.9	Unconcn stool; 1% basic fuchsin; confirm with S-MB	Children (high); all cases in <2 to 5 yr old; malnourished more symp, sicker; only pathogen in 25 pos stools; 2 deaths; definite link to malnutrition	196
	United States (Oklahoma)	186	142	24.6	F-Eth Acet concn; mod Kinyoun acid fast	Day-care center outbreak; 35% pos (symp), 12% pos/asymp; <3 yr (high); 142/186 stool exams; 23% contacts pos; 2% no contacts pos	131
	Sweden	780	780	1.0	Stool concn, mod Ziehl-Neelsen	Patients submitting stools for routine ovum and parasite exams; 19/29 recently traveled abroad	16
	Sweden	698	698	3.0		All patients acute gastroenteritis, hospitalized	16
	South Africa	194	194	15.5	Mod acid fast	All symp children; in hospital with cryptosporidiosis = significantly higher mortality	351
	United Kingdom	742	742	6.0	S-MB	All symp children; most >2 yr old; 89% watery diarrhea, 80% vomiting	313
	Australia	2,248	2,248	2.5	Stool unconcn; mod acid fast (cold)	45% of pos specimens were formed stools; common in warm and dry months; most common age <10 (31% pos); person-to-person transmission	30
	Thailand	NR	1,500	0.5	Mod acid fast	Children, adults: children with diarrhea (3.7%); stools no WBCs; acute diarrhea main symp; common cause of nonviral diarrhea in young children	311
	West Africa	NR	270	3.7	S-MB	Children <5 yr old; 12.5% with diarrhea pos, 1.8% pos asymp; age 7–12 mo (high); dry season	49
	Germany	470	1,160	1.1	Carbolfuchsin rapid neg; S-MB; Giemsa, mod Ziehl-Neelsen; methylene blue + acid fast	Both immunocompetent and immunosuppressed with and without diarrhea; *Cryptosporidium* should be considered in patients with diarrhea	159
	Switzerland	2,367	2,367	1.4	Auramine; mod Ziehl-Neelsen	Aug and Sept more common; children (high)	198
	France	235	235	3.8	Mod Ziehl-Neelsen	Children; healthy carriers 2.2%; emphasizes existence of healthy carriers	172
	Bangladesh	2,056	2,056	3.5	Stool concn; mod acid fast (hot), KOH; confirmed with Giemsa	Children; <2 yr (high/73%); peak Apr/July; histology discussed; increased cell turnover	280
	Chile	750	750	6.4	Mod acid fast/hot	All symp; <4 yr old (mean age, 14.7 mo); 144 asymp (all neg.); 2 yr olds (high); 96% acute diarrhea; autumn-winter (high)	346
1988	Guatemala	130	1,280	15.4	KOH concn, S-MB	Infants 0–11 mo; Feb–May (high-end of dry season); contaminated weaning foods, animals; poor hygiene cited	70
	United States (Ohio)	NR	2,780	0.3	F-Eth Acet concn, Kinyoun acid fast/cold	Plus 912 biopsies (all neg); recommendation: screen immunosuppressed or those with persistent diarrhea; support geographic variation in prevalence	123
	Jerusalem	221	221	13.5	No concn, safranin; confirm pos (Giemsa)	Children; symp; most common pathogen; malnourished children/longer illness; suggest important interaction between diarrhea and malnutrition	273

Continued on following page

TABLE 2—*Continued*

Yr	Location	No.[a]		% Positive	Diagnostic method[b]	Comment(s)[c]	Reference
		Patients	Specimens				
	United Kingdom	234	234	11.0		All AIDS patients; 1–6 specimens; avg no. of specimens needed for dx = 3; use of zidovudine (AZT) discussed (3 patients reported *Cryptosporidium* no longer found in stool)	64
	Nepal	328	328	5.0	DMSO, mod acid fast	All symp, diarrhea; *Blastocystis hominis* in 33%; traveler's diarrhea	310
	San Salvador	210	420	3.8	MIF; auramine, mod Ziehl-Neelsen	Children <2 yr (high)	261
	South Africa	92	17	18.4	Mod Kinyoun acid fast	All children; all had diarrhea; considered important cause of diarrhea	29
	Australia (North Queensland)	780	780	4.6	Kinyoun acid fast	All symp, immunocompetent; <5 yr old, 25–33 age groups (high); 3rd most common organism after rotavirus and *Giardia*; no seasonal variation; exam for this organism warranted in symp patients	69
	United States (North Carolina)	10	10	100.0		All symp; veterinary students; direct exposure to infected calves and contaminated material; diarrhea 80%	181
	Africa (Zaire)	42	42	30.0		AIDS patients with persistent diarrhea; 12% *I. belli*; etiology of persistent diarrhea in most African AIDS patients still unclear; discussion of endoscopy and histological findings	60
	Italy	232	232	0.86	Mod Teleman-Miyagawa concn; DMSO acid fast	Two pos patients <2 yr old; conclusion indicates, in spite of low incidence, screening compromised patients justified	228
	Scotland					83 pos cases over 2 yr (58 children, 25 adults); spring/autumn peaks; diarrhea, vomiting common; important cause of traveller's diarrhea; incubation 2–11 days	282
	Scotland	49	>49	100.0		*Cryptosporidium* found up to 35 days after onset of symp (most stopped at 20 days); 76% symp corresponded to shedding period	281
	Saudi Arabia	321	321	1.0		Pos were children, 2 yr old; 4% *Giardia*	160
	Portugal	104	104	27.0		Day-care center; most symp with watery diarrhea; assoc with *Giardia* not significant; person-to-person transmission suggested	217
	South Africa	90	90	73, 10		73% of children, 10% adults pos; shedding persisted up to 50 days; person-to-person transmission suggested	341
1989	United States (New Mexico)	78	78	100.0		All ages (median, 3 yr); strong association with drinking surface water and illness; also in children assoc with day-care center where other children were ill	107
	United States (California)	1,516	2,786	0.86	F-Eth Acet concn, monoclonal FA antibody	All ages; all stools submitted for ova and parasite exams; univ med center setting	108
	United States (New York)	400	400	0.5	F-E concn; saline/iodine wet mounts; rhodamine-auramine O, monoclonal FA antibody	Low pos rate in nonrisk populations argues against routine testing and/or use of expensive reagents	20

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TABLE 2—*Continued*

Yr	Location	No.[a]		% Positive	Diagnostic method[b]	Comment(s)[c]	Reference
		Patients	Specimens				
	Peru	153	153	None given		All symp infants; diarrhea associated with several organisms, including *Cryptosporidium*; contaminated weaning foods implicated	32
	South Africa	NR	NR	None given		Children (3-yr study); *Cryptosporidium* and *Giardia* most common parasites (all ages of children)	299
	France	132	132	21.2		All AIDS patients with pathogens (other organisms recovered) had diarrhea; endoscopy recommended as adjunct to stool exams; most common pathogen assoc with diarrhea = *Cryptosporidium*	263
	Spain	699	699	1.1		All pos children (immunocompetent); 75% diarrhea, 50% vomiting; 5/8 cases Jan–Apr; 7/8 in children <3 yr old	115
	United States (Georgia)	147	147	39.0	Monoclonal FA antibody	Waterborne outbreak; conclusion: current standards for treatment of public drinking water may not prevent contamination with *Cryptosporidium*; all patients had gastroenteritis	129
	Switzerland	210	390	2.4		Children, all symp, immunocompetent; watery diarrhea, vomiting; marked compensated metabolic acidosis; recommend screening for both *Cryptosporidium* and *Aeromonas* in gastroenteritis in children	90
	Egypt	151	151	9.0		Mean age, 18 mo; all symp with diarrhea; potential source of infection was clay water storage containers	219
	United States (Texas)	46	46	58.7		Day-care center outbreak; children (34), adults (12); 55% had diarrhea; person-to-person transmission	237
	England	36	NR	100.0		3/68 pos stools 38 days after onset of symp; person-to-person transmission suggested; no evidence of waterborne spread	45
	India	77	NR	13.0		All <8 yr, symp with diarrhea; no excretors in group of 155 controls, no diarrhea; 14.3% of children <2 yr	307
	Italy	124	NR	7.2		All symp with diarrhea (2–30 days); mean age, 34 mo; all cases in warm season; 3rd most common pathogen after rotavirus and *Salmonella*	47
	Argentina	210	300	7.6	F-E concn; 1% safranin; mod Ziehl-Neelsen	One wk to 13 yr; all symp with diarrhea; most <3 yr	356
	Australia	12	NR	19.0		19% included *Cryptosporidium* and *Giardia*; all 12 were children undergoing bone marrow transplants	36
	Ireland	1,621	NR	4.0		All <14 yr; symp with diarrhea; most pos from rural background; peak cases in late winter (summer, early autumn in 1981); LOS dropped from 18.3 (1981) to 9.5 (1987)	48
	United States (Colorado)	30	150	30.0	Sedimentation concn; mod acid fast	All ≤5 yr; 6 asymp (5 <2 yr); 2 <2 yr symp with diarrhea	92

Continued on following page

TABLE 2—*Continued*

Yr	Location	No.[a]		% Positive	Diagnostic method[b]	Comment(s)[c]	Reference
		Patients	Specimens				
	Saudi Arabia (Kuwait)	10	NR	100.0	F-Eth Acet concn; direct smears S-MB; neg staining of trichrome "clue"; destained, restained with S-MB	All children symp with diarrhea, fever, vomiting, dehydration	138
	Mexico	30	NR	16.7		All children; 6/30 symp; all AIDS; other frequent infections *Candida*, pneumonia, sepsis, UTI, otitis	17
	Africa (Burundi)	100	100	15.0	Stool exam plus duodenal aspirate	All AIDS patients; *Isospora*, 20%; *Strongyloides*, 10%	105
	China	NR	1,014	0.5	Mod acid fast; S-MB; auramine-phenol	Specimens collected from 4 hospitals in Nanjing; oocysts difficult to find on S-MB (especially if numbers are low)	122
	Egypt	213	NR	3.2	Mod Ziehl-Neelsen; S-MB used for confirmation	All infants and children; all symp with diarrhea	161
	Spain	1,973	1,973	1.5	Monoclonal FA antibody; Ziehl-Neelsen	Children (1.4%); adults (2.2%); 55.5% of pos in children <4 yr; higher incidence in winter, spring; diarrhea, abdominal pain most common; asymp carriers found in both children and adults	114
	Africa (Burundi)	100	NR	13.1		All AIDS; 84/100 diarrhea; *Isospora*, 16.2%	154
	Africa (Ivory Coast)	104	NR	9.0		All hospitalized with diarrhea; acute diarrhea, vomiting, hyperthermia; 20% HIV Ab pos	169
	United States (New York)	169	380	6.0		12.7% duodenal aspirates; no patients had diarrhea, no pos duodenal biopsies; high asymp carrier rate	266
	Cuba	200	600	8.0	Direct wet mount, F-E concn, mod Ziehl-Neelsen	Numbers second only to *Giardia* (10%); 13/16 only parasite; more common in young children; all pos were bottle fed	88
	Netherlands	NR	2,000	1.2	Safranin, mod Ziehl-Neelsen	Liquid stools (1.86%), formed (0.89%); highest in 1–10 and 51–60 yr, June, Aug, Dec; 160 no diarrhea (all neg); screening not recommended	19
	India	180	NR	4.4		All 180 admitted to hospital with acute gastroenteritis	247
		100	NR	0.0		100 normals	
	South Africa	373	NR	6.0		Same incidence in symp patients (373) as in normal controls (371); role of "home remedies," complex nature of diarrhea in developing countries discussed	185
		371	NR	6.0			
	Africa (Kenya)	846	1,420	3.8	Mod Ziehl-Neelsen	All children 0–60 mo; 320 controls (no diarrhea, same age) all neg; infection assoc with acute childhood diarrhea	284
	South Africa	3,186	NR	4.1		Adults and children, all caucasian; most infections in children <5 yr (6.2%); increase in Jan–May	300
	United States (South Dakota)	247	NR	1.6		All children	233
	Africa (Kenyatta)	NR	133	3.8		All loose or diarrheic stools	96
	India (Calcutta)	566	NR	5.6		Highest in 0–6 mo; watery stools, diarrhea <7 days; higher in monsoon, post-monsoon months	241
		167	NR	1.2			

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TABLE 2—*Continued*

Yr	Location	No.[a]		% Positive	Diagnostic method[b]	Comment(s)[c]	Reference
		Patients	Specimens				
	Chile	NR	1,039	3.7	Ziehl-Neelsen	8.5% among malnourished, 1.9% among ambulatory patients; highest among milk-feeding infants	234
	India	266 294	560	4.5	Phenol-auramine (FA), mod. Ziehl-Neelsen	Children 2 wk–10 yr; 6% among 266 symp, 3% among 294 controls	262
	Ivory Coast	148	NR	6.7		Adults, chronic diarrhea, suspected HIV pos	312
1990	United States (Oregon)	5,256	NR	1.1		Young children higher incidence	285
	Africa (Zambia)	63 36	NR NR	32.0 0.0		63 HIV seropos; 36 HIV seroneg; villous blunting, inflammation	63
	Bangladesh	1,382 235	NR NR	3.0 0.0		31/42 no other pathogens; higher in children <5 yr; more cases in Apr–July; index cases excreted oocysts 3–28 days	255
	England, Wales	16,421	NR	0.5–3.9		Incidence highest in children 1–4 yr; abdominal cramps, watery diarrhea; 12% acquired abroad, 9% drank raw milk, 22% close contact with farm animals; 1 nursery school outbreak	254
	Brazil	61	201	5.2	Mod Ziehl-Neelsen	Children 1–2 yr; 5.2% of symp, asymp neg; self-limited in immunocompetent children	189
	Switzerland	455	910	4.6	F-E concn, mod Ziehl-Neelsen	All children; respiratory symptoms more common in pos patients (42%, 13% controls); person-to-person transmission	94
	Venezuela	320	>600	4.8		Children 1–10 mo; rarely detected if child did not have diarrhea	246
	Israel	1,073	NR	7.7		Children; more common <5 yr; diarrhea 85%; recovery 5–9 days	223

[a] If the numbers of patients = the number of specimens, only one stool specimen per patient was examined. NR, Not reported.
[b] F-E concn, Formalin-ether concentration procedure; F-Eth Acet, formalin-ethyl acetate concentration procedure; MIF, merthiolate-iodine-formalin; Mod, modified; DMSO, dimethyl sulfoxide; S-MB, safranin-methylene blue.
[c] Comments in parentheses are used to compare percent positive samples of different age groups. Assoc, Associated; Symp, symptomatic or symptoms; Asymp, asymptomatic; Dx or dx, diagnosis; pos, positive; neg, negative; LOS, length of stay; UTI, urinary tract infection; WBC, leukocytes.

Cryptosporidium oocysts in stool specimens, from at least 40 countries, is presented in Table 2. Data from all of these surveys, excluding documented outbreaks, indicate that in the more industrialized countries of North America and Europe the prevalence rate is between 1 and 3%. In contrast, mean prevalence rates are higher in underdeveloped continents, ranging from approximately 5% in Asia to approximately 10% in Africa. The higher prevalence in underdeveloped countries may be due to the lack of clean water and sanitary facilities, crowded housing conditions, and large numbers of potential reservoir hosts (domestic mammals) near homes. In general, it appears that cryptosporidiosis is more common in crowded urban areas in developing countries than in less crowded rural areas. The reverse appears to be true in more developed countries.

Estimates provided by Walsh and Warren (340) suggest that in Asia, Africa, and Latin America alone there are as many as 5 billion episodes of diarrhea and 5 to 10 million diarrhea-associated deaths annually. If these estimates are accurate and if the *Cryptosporidium* prevalence data summarized above are correct, then one may predict 250 to 500 million *Cryptosporidium* infections annually in persons living in Asia, Africa, and Latin America.

Seroprevalence. Limited serologic surveys also support the concept that *Cryptosporidium* infection is more common in developing countries compared with the more industrialized regions of North America and Europe. Seroprevalence rates in Europe and North America are usually between 25 and 35% (51, 129, 167). In contrast, approximately 64% of 389 children and adults in Lima, Peru, and 64% of 84 children in Maracaibo and Caracas, Venezuela, had serologic evidence of previous infection; i.e., their sera contained antibodies (immunoglobulin G [IgG] and/or IgM) specific for *Cryptosporidium* spp. (332). At the beginning of a longitudinal serologic survey (333), of 56 United States Peace Corps volunteers in Africa, 15 (26.8%) were seropositive. During the next year an additional eight (14% of the 56) seroconverted. A similar rate of seroconversion occurred

during the second year. These data suggest that *Cryptosporidium* infections may be more common in most regions than fecal oocyst surveys have indicated. They also point out the increased risk of infection when previously unexposed persons travel or work in areas of high prevalence.

The epidemiologic features of cryptosporidiosis emphasized above i.e., transmission by environmentally resistant cysts (oocysts), existence of numerous potential reservoir hosts for zoonotic transmission, documentation of person-to-person transmission in settings such as day-care centers, occurrence of asymptomatic infections, and ubiquitous environmental distribution resulting in the likelihood of waterborne transmission, are similar to those of human giardiasis revealed during the past decade. *C. parvum* is now gaining the recognition it deserves as an important, widespread cause of diarrheal illness in humans. In light of the epidemiologic information reviewed, it is important that health care professionals emphasize the importance of *Cryptosporidium* in training programs so that cryptosporidiosis is considered in the differential diagnosis of diarrheal illness. This educational role should be approached aggressively because of the common occurrence of the disease, because of the large number of potential reservoir hosts, and because persons with impaired immune function may develop life-threatening cryptosporidiosis.

Prevalence of HIV-infected persons. At present, there are not enough valid data to provide an accurate assessment of the prevalence of cryptosporidiosis in AIDS patients. Data based on physician reporting of diagnosed cases of cryptosporidiosis to the Centers for Disease Control (CDC) have resulted in an estimated prevalence of 2 to 5% for late-stage human immunodeficiency virus (HIV)-infected patients in the United States. As of 4 April 1986, 3.6% (697 of 19,182) of AIDS patients reported to the CDC had been diagnosed with cryptosporidiosis (231). A later statistic reveals that 3.1% of the 30,632 cases of AIDS reported to the CDC as of 7 February 1987 were diagnosed as having cryptosporidiosis. More recent reports indicate that data provided by the CDC are an underestimation (278). In patients with AIDS and diarrhea, 15% of those evaluated at the National Institutes of Health in Bethesda, Md., and 16% of those evaluated at the Johns Hopkins Hospital in Baltimore, Md., were infected with *Cryptosporidium*, the most common enteropathogen in the latter study (175, 293). In one hospital in Great Britain, 11% of AIDS patients had cryptosporidiosis (64). Of the *Cryptosporidium*-positive patients in Great Britain, 19% were thought to have died as a direct result of cryptosporidiosis. In a study from France, 21.2% of 132 AIDS patients had cryptosporidiosis (263).

Since cryptosporidiosis is more prevalent among immunocompetent persons in developing countries compared with those in industrialized countries, one may predict that a similar difference exists in the AIDS population. One study reported that 27 of 29 AIDS patients from Haiti had chronic diarrhea and that 41% (11 of 27) had *Cryptosporidium*-positive stools (199). In one report from Kinshasa, Zaire, 85% (109 of 128) of the patients presenting with diarrhea of over 1-month duration were HIV seropositive and 22% of 106 of these patients that were studied were stool positive for *Cryptosporidium* (135). Other data from Africa indicated *Cryptosporidium*-positive rates in AIDS patients of 15% (105) and 13.1% (154). One study from a hospital in Brazil reported that 12% of the AIDS patients with diarrhea had *Cryptosporidium*-positive stools (91). Another report from Mexico indicated that 16.7% of children with AIDS had cryptosporidiosis (17). The overall prevalence of intestinal and extraintestinal cryptosporidiosis in AIDS patients residing in industrialized and developing countries remains unclear and requires additional studies with proper diagnostic techniques.

CLINICAL FEATURES

The most common clinical feature of cryptosporidiosis in immunocompetent and immunocompromised persons is diarrhea, the symptom that most often leads to diagnosis. Characteristically, the diarrhea is profuse and watery; it may contain mucus, but rarely blood and leukocytes, and it is often associated with weight loss. Other less common clinical features include abdominal pain, nausea and vomiting, and low-grade fever (<39°C). Occasionally, nonspecific symptoms such as myalgia, weakness, malaise, headache, and anorexia occur. The severity of these symptoms may wax and wane in individuals and often parallels the intensity of oocyst shedding. Both the duration of symptoms and the outcome typically vary according to the immune status of the host. AIDS patients usually experience a prolonged, life-threatening illness, whereas most immunocompetent persons experience a short-term illness with complete, spontaneous recovery. However, the clinical presentation of gastrointestinal cryptosporidiosis does not always fit one of these two divergent categories. Persons with the clinical and laboratory features of AIDS have been reported to clear infections after several months of diarrhea, and individuals reported to be immunocompetent have had infections lasting more than 1 month (73, 167). Asymptomatic infections have been reported in immunocompetent persons (69) and in one patient with AIDS (355).

Immunocompetent Persons

Most of the 18 cases of cryptosporidiosis in immunocompetent humans reported prior to 1983 (7, 18, 55, 104, 216, 236, 257, 326) and the numerous cases reported since then (see Table 2 for references) describe a self-limited, cholera-like or flulike gastrointestinal illness. The most common symptoms reported are profuse, watery diarrhea (cholera-like) and abdominal cramping, nausea and vomiting, low-grade fever, and headache (flulike). After reviewing the symptoms reported for 586 persons in 36 large-scale surveys, Fayer and Ungar (101) reported that diarrhea was the most commonly listed clinical feature (92%), followed by nausea and vomiting (51%), abdominal pain (45%), and low-grade fever (36%). In most well-nourished persons, diarrheal illness due to *C. parvum* infections lasts from 3 to 12 days. Occasionally, these patients may require fluid replacement therapy, and occasionally the diarrheal illness may last for more than 2 weeks. In poorly nourished children with cryptosporidiosis, oral and parenteral rehydration therapy is often required because of excessive fluid loss that may last more than 3 weeks.

Failure-to-thrive has been reported in infants either as a result of or as a factor contributing to persistent cryptosporidiosis (126, 127, 145, 173, 274, 313). Malnutrition may contribute to increased length of diarrheal illness, hospitalization, and perhaps to fatality associated with intestinal cryptosporidiosis (39, 158, 196, 234, 273, 289, 309, 350, 351). For example, one study (273) from a hospital in Jerusalem revealed that children with diarrhea and *Cryptosporidium*-positive stools were significantly more malnourished than children with diarrhea and no *Cryptosporidium* oocysts in their stools. Also, children with severe malnutrition and with

Cryptosporidium oocysts in their stools had a significantly longer duration of diarrhea than similarly malnourished children without cryptosporidiosis. Diarrheal illness is a major cause of morbidity and mortality, especially in young children living in developing countries. On the basis of limited prevalence data from stool and serologic surveys (reviewed above) and the increasing number of reports associating cryptosporidiosis and malnutrition, it is likely that *Cryptosporidium* plays an important role in the overall health status of these children. It is possible that *Cryptosporidium* may also play a role in respiratory disease that often accompanies diarrheal illness in malnourished children (94). Data supporting the later concept await properly conducted studies.

Immunodeficient Persons

Intestinal cryptosporidiosis. Typically, the duration of diarrheal illness and ultimate outcome of intestinal cryptosporidiosis depend on the immune status of the patient. In the most severely immunocompromised host, such as persons with AIDS, diarrheal illness due to *Cryptosporidium* infection of the gastrointestinal tract becomes progressively worse with time and may be a major factor leading to death. It is believed that the infection usually begins with organisms colonizing the ileum or jejunum and develops into a life-threatening condition when a large portion of the gastrointestinal mucosa is covered with parasites (40, 288). Fluid loss in patients with AIDS and cryptosporidiosis is often excessive; 3 to 6 liters of diarrheic stool per day is common, and as much as 17 liters of watery stool per day has been reported (55). Numerous case reports of intestinal cryptosporidiosis in AIDS patients can be found in the literature (see Table 2 for references).

In patients with other immune deficiencies, length and severity of illness may depend on the ability to reverse the immunosuppression. Patients included here are those on immunosuppressive chemotherapy, especially for cancer and transplantation (61, 162, 183, 214, 216, 221, 238, 268, 306, 345); malnourished individuals, particularly children (39, 49, 70, 126, 158, 196, 205, 206, 261, 273, 279, 280, 289, 309, 313, 347, 348); and persons with concurrent viral infections such as measles, chicken pox, or cytomegalovirus (38, 89, 125, 145, 261, 301, 309, 344).

In the immune deficient patient, *C. parvum* infections are not always confined to the gastrointestinal tract, and additional clinical symptoms have been associated with these extraintestinal infections. These symptoms include a variety of respiratory problems, cholecystitis, hepatitis, and pancreatitis.

Respiratory cryptosporidiosis. The number of case reports of *Cryptosporidium* infection of the respiratory tract is growing rapidly (41, 94, 106, 119, 125, 139, 162, 168, 195, 200, 222, 348). The symptoms associated with these infections include cough, shortness of breath, wheezing, croup, and hoarseness. Diarrhea has not been reported in all of these patients. Oocysts have been identified in sputum samples, tracheal aspirates, bronchoalveolar lavage fluid, brush biopsy specimens, and alveolar exudate obtained from lung biopsy. *Cryptosporidium* sp. has been the only pathogen isolated from at least four HIV-infected patients (139); however, concurrent pulmonary infections with cytomegalovirus, *Pneumocystis carinii*, or *Mycobacterium* spp. have been reported in most cases. Most patients with severe immune deficiencies and *Cryptosporidium* sp. in their respiratory tract do not recover.

Cryptosporidium sp. has also been documented as the cause of acute laryngotracheitis in an infant (125). In children, severe intestinal infections with *Cryptosporidium* sp. have been associated with the acute phase of measles, a cause of transient immunosuppression (39, 89, 261). The role of *C. parvum* as a common cause of diarrheal illness in immunocompetent persons and in persons whose immune function is compromised because of congenital or acquired immune deficiencies or because of malnutrition and/or other infectious diseases is well established (43, 69, 131, 158, 196, 279, 280, 289, 330); however, its importance as a cause of respiratory illness remains to be determined.

Gallbladder and biliary tree cryptosporidiosis. At present, gallbladder disease, primarily acalculous cholecystitis and, less frequently, sclerosing cholangitis, has been reported in approximately 12 HIV-infected patients (38, 120, 121, 136, 155, 202, 249, 276). Symptoms most often reported include fever, right upper quadrant nonradiating pain, nausea, vomiting, and simultaneous diarrhea. Jaundice may also occur, and alkaline phosphatase and bilirubin have been elevated whenever measured. The gallbladder and common bile duct are usually enlarged and have thick walls, and in cases of common bile duct stenosis the associated extrahepatic ducts are usually dilated. Diagnosis has generally been made by histologic examination of the gallbladder epithelium or by the demonstration of oocysts in bile. Oocysts are not always found in the feces, especially in cases of common bile duct stenosis that results in little or no release of bile into the intestine. Developmental stages of *Cryptosporidium* sp. have also been identified in bile duct epithelium in liver biopsies obtained from several patients with cholecystitis (120, 155, 202). Hepatitis with elevated liver enzymes was reported in one of these patients (120).

Pancreatic duct cryptosporidiosis. Several cases of symptomatic pancreatitis and concurrent cryptosporidiosis have been reported (120, 128, 136, 155, 168). In one reported case, an immunocompetent 14-year-old farm girl had severe abdominal pain and a serum amylase level of 14,000 U/liter (normal, <300 U/liter) 1 week after diagnosis of cryptosporidial enteritis. Extensive workup showed an enlarged pancreas with ascites and no other etiology. Her symptoms resolved spontaneously over 6 weeks (128). In one published case, *Cryptosporidium* oocysts were found at necropsy in the pancreatic ducts of a child with severe combined immune deficiency (168). Two AIDS patients with cryptosporidiosis had pancreatitis accompanying cholecystitis (136, 155). Endogenous stages of *Cryptosporidium* sp. have also been found in epithelial cells lining pancreatic ducts of nonhuman primates (several species) infected with simian immunodeficiency virus or a type D retrovirus (21, 180).

PATHOGENICITY

At present, the pathophysiologic mechanisms of *Cryptosporidium*-induced diarrhea are poorly defined. Studies in germfree calves monoinfected with *C. parvum* suggest that malabsorption and impaired digestion in the small bowel coupled with malabsorption in the large intestine are major factors responsible for diarrhea in calves with cryptosporidiosis (133). Similar malabsorption, attributed to parasite-induced villous damage, has also been reported in a neonatal pig model (12a). This malabsorption and impaired digestion may result in an overgrowth of intestinal microflora, a change in osmotic pressure across the gut wall, and an influx of fluid into the lumen of the intestine. Malabsorption and

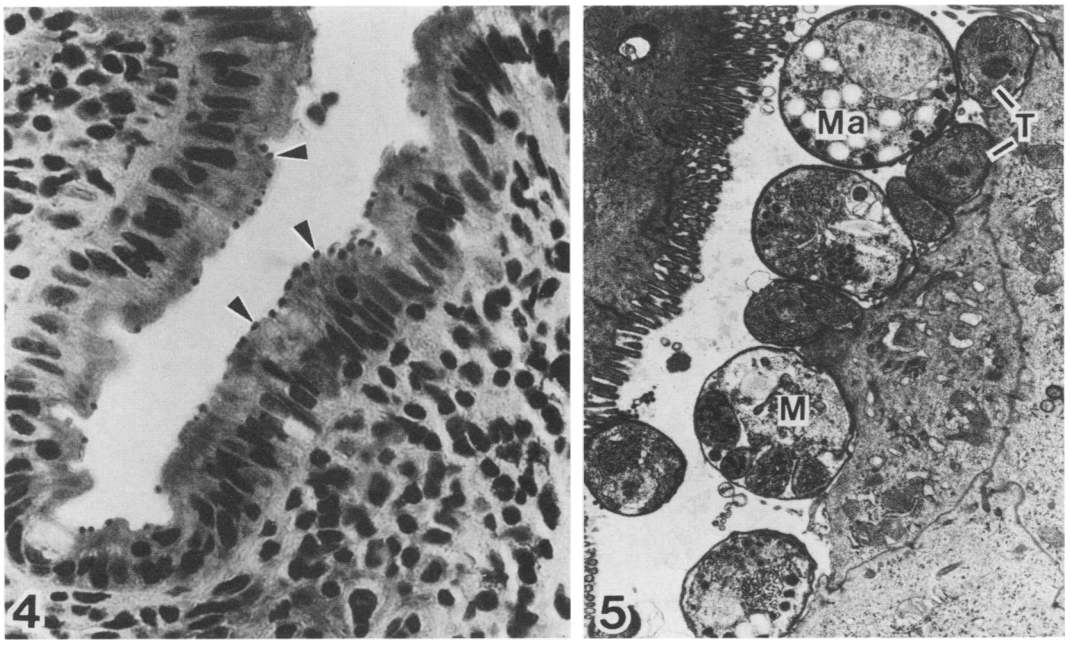

FIG. 4. Light photomicrograph of a histologic section (stained with hematoxylin and eosin) of a small bowel biopsy obtained from an immunocompromised patient with persistent cryptosporidiosis. Three of the numerous development stages of *C. parvum* within the brush border of the enterocytes are denoted by arrowheads.

FIG. 5. Transmission electron micrograph of developmental stages of *C. parvum* within parasitophorous vacules bulging from the microvillous region of ileal enterocytes of an experimentally infected mouse. Macrogametes (one labeled Ma) contain the characteristic amylopectin granules near the center and wall-forming bodies near the periphery. Several (two labeled T) trophozoites (uninucleate meronts) and one meront (M) with budding merozoites can be seen.

impaired digestion have also been reported in humans infected with *C. parvum*. The secretory (often described as choleralike) diarrhea common to most immune deficient patients with cryptosporidiosis suggests a toxin-mediated hypersecretion into the gut; however, we are not aware of reports documenting such a toxin. Definitive systematic studies are needed to determine the mechanisms by which *C. parvum* and its metabolites or toxins may alter normal intestinal function of a susceptible animal model.

DIAGNOSIS

Histologic Diagnosis

Prior to 1980, human cryptosporidiosis was diagnosed histologically by finding the small spherical life cycle stages of *C. parvum* in the microvillous region of the intestinal mucosa obtained by biopsy or in tissue obtained at necropsy (12). In hematoxylin-and-eosin-stained sections, developmental stages of the parasite appear as small, spherical, basophilic bodies (2 to 5 μm depending on stage of life cycle) within the microvillous region of the intestinal mucosa (Fig. 4). Transmission electron microscopy can be used to confirm diagnosis and reveals distinct life cycle forms, each within a parasitophorous vacuole confined to the microvillous region of the host cell (Fig. 5). The location of these parasites has been described as intracellular-extracytoplasmic: intracellular because they reside within a parasitophorous vacuole, extracytoplasmic because they are confined to the microvillous region of the host cell (81, 117). Special staining procedures have not provided marked improvements over routine hematoxylin and eosin for histologic diagnosis. The need for invasive procedures and for rapid fixation and careful processing to avoid loss of organisms from the microvillous border are problems associated with biopsy specimens. Also, because of the organism's size and the fact that not all regions of the intestinal tract are infected, sampling errors often occur. Such invasive, expensive, and time-consuming procedures are no longer required for diagnosis since a variety of techniques have been developed to identify *C. parvum* oocysts in fecal specimens, sputum, and bile. However, the use of biopsy specimens and light or electron microscopy may be of value to investigate such aspects as the histopathology and cytoarchitectural changes associated with infection.

Cryptosporidium oocysts can be identified in paraffin-embedded tissue sections by indirect immunofluorescent-antibody (IFA) techniques, using *Cryptosporidium*-specific monoclonal antibodies (15, 186).

Laboratory Diagnosis

Since *Cryptosporidium* infections of a mucosal epithelium result in the release of numerous oocysts, specimens of stool, sputum, or bile represent a sampling of the entire intestinal, respiratory, or biliary tract. These specimens can be evaluated by a variety of diagnostic procedures to identify the environmentally resistant *Cryptosporidium* oocyst (9, 22, 24, 26, 44, 68, 71, 82, 108, 112, 116, 142, 156, 194, 218, 250, 251). For the diagnosis of cryptosporidiosis, stool and other body fluid specimens should be submitted as fresh material or in 10% formalin or sodium acetate-acetic acid-formalin (SAF) preservatives. Fixed specimens are recommended because of biohazard considerations. Potassium dichromate

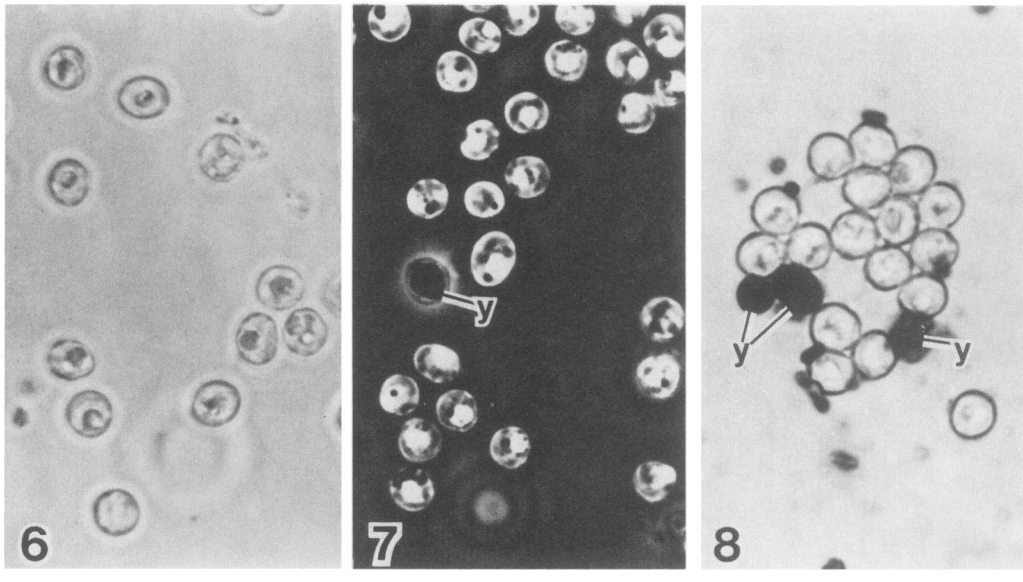

FIG. 6. Oocysts of *C. parvum* as seen with bright-field microscopy following concentration by Sheather's sugar flotation (4). With some bright-field optics, oocysts often appear light pink.

FIG. 7. Oocysts of *C. parvum* as seen with phase-contrast microscopy following concentration by Sheather's sugar flotation (82). Oocysts appear as bright birefringent spherical bodies against a dark background. Oocysts also contain one to four dark granules. Yeast cells (y) are not bright and birefringent.

FIG. 8. Oocysts of *C. parvum* as seen with bright-field microscopy following negative staining with carbolfuchsin (68). Fecal debris and yeast cells (y) stain darkly, whereas the stain surrounds but does not penetrate the wall of the oocyst. Because the negative-stained preparation is covered with immersion oil, and because the interior of oocyst wall contains water, oocysts appear bright and birefringent.

solution (2 to 3% [wt/vol] in water) is used routinely as a storage medium to preserve oocyst viability; it is not a fixative. Fresh or preserved stool specimens can then be examined by several concentration or staining procedures which aid in the visualization of *Cryptosporidium* oocysts.

The number of oocysts shed in stool may fluctuate (4, 35, 111, 112, 153, 302, 342); therefore, it has been recommended that a minimum of three specimens be collected, the same recommendation as for routine ovum and parasite examination (O&P) (64, 111). Multiple samples are particularly important when dealing with formed stool specimens, which usually contain fewer oocysts than do diarrheic specimens.

Various screening approaches for clinical specimens have been recommended, and they vary from screening every stool specimen for *Cryptosporidium* oocysts to screening only very selected risk groups (22, 27, 47, 50, 52, 62, 69, 90, 130, 159, 203, 228). Although prevalence values tend to be low unless specific risk groups are screened (109), there are a number of options to consider. A comprehensive screening approach would be to examine specimens from all symptomatic patients (69). However, considering cost containment issues, a selective approach might be more reasonable. Screening all patients with diarrhea has been recommended (50, 69, 159, 203). All compromised patients could be screened (228), or a more limited approach could be to screen immunosuppressed patients with diarrhea (46, 203). Others think that all children with gastroenteritis should be screened (27, 62, 90, 130). Another option would be to perform diagnostic procedures for the diagnosis of cryptosporidiosis only when specifically requested to do so. The approach for each laboratory will vary depending on the patient population served, physician ordering patterns, availability of technical personnel, cost containment needs, and overall clinical relevance of the test results.

Although there is some evidence that regional and seasonal variability may occur with cryptosporidiosis, this possibility would probably not affect the laboratory's overall approach to specimen acceptance for diagnostic testing (30, 47–49, 70, 198, 201, 224, 241, 255, 280, 282, 346).

Concentration techniques. Stool concentration techniques include flotation of oocysts in Sheather's sugar solution, in zinc sulfate (1.18 or 1.20 specific gravity), or in saturated sodium chloride (1.27 specific gravity). Stool concentration techniques using sedimentation include formalin-ether and formalin-ethyl acetate. Some workers have found no differences among these methods, whereas others have found the formalin-ether and sodium chloride flotations to be the most sensitive (5, 53, 112). Some researchers think that the Sheather's sugar solution gives results equal to or better than those obtained with formalin-ether or formalin-ethyl acetate (88, 213, 357). When Sheather's sugar solution is used, oocysts appear pink-tinged by bright-field microscopy (Fig. 6) and bright and birefringent when viewed by phase-contrast microscopy; yeast cells are not pink or bright and birefringent (Fig. 7). When left in Sheather's solution for >15 min, oocysts begin to collapse and lose their spherical shape. It is important to consider that most sedimentation techniques used in the clinical microbiology laboratory were designed for the diagnosis of helminth eggs and protozoan cysts (e.g., *G. lamblia* and *Entamoeba* spp.) that are larger than *Cryptosporidium* sp. Therefore, after the short centrifugation times at low *g*-force (300 × *g* for 2 min), many of the oocysts may remain in the supernatant. If one is looking for

C. parvum oocysts in stool or other body fluid samples, it is advisable to centrifuge at >500 × *g* for at least 10 min.

Staining techniques. Most recommended stains for *Cryptosporidium* oocysts cannot be performed on stools preserved in polyvinyl alcohol (PVA) fixative. The routine stains (trichrome and iron hematoxylin) used for stool diagnosis of other parasites are not acceptable for the identification of *Cryptosporidium* oocysts (110, 134a, 174, 216, 302). Several widely used techniques for demonstrating *Cryptosporidium* oocysts in fecal specimens from humans and other animals are modified acid-fast staining (112, 194, 251), negative staining (Fig. 8) (71, 250), and Sheather's sugar flotation (Fig. 6 and 7) (82, 257). Although the last two procedures are useful in the research laboratory, acid-fast staining is usually the method of choice for the clinical microbiology laboratory. Some modifications have incorporated dimethyl sulfoxide (3, 44, 251). In any of the acid-fast methods, there may be some variability in stain uptake, related to the stain itself or the age of the oocysts after prolonged storage (24, 53, 112, 235). Acid-fast stains can also be performed by using either the hot staining method (112) or the cold method (193, 194). Less common staining methods that have been used are cited in Table 2 and in a recent review (113).

Fluorescent stains for demonstrating *Cryptosporidium* oocysts include auramine-rhodamine (112, 194, 244), auramine-carbolfuchsin (52, 66), and acridine orange (112, 194). However, confirmatory staining of suspected oocysts by another method may be required (52, 112). All stained preparations should be examined with high-dry or oil immersion lenses for routine screening and confirmation.

Considerable experience with the concentration and staining methods is often required to obtain an accurate diagnosis. For this reason, IFA procedures with *Cryptosporidium*-specific polyclonal or monoclonal antibodies have been developed to aid in the identification of oocysts in stool specimens (15, 108, 211, 239, 303). This approach may provide the most sensitive method available for the diagnosis of cryptosporidiosis (15, 20, 108).

Serodiagnosis. The use of serodiagnostic techniques to monitor exposure to *Cryptosporidium* sp. has thus far been limited to a few laboratories. Antibodies specific to *Cryptosporidium* sp. have been detected by an IFA procedure in sera obtained from persons who recovered from confirmed infections (46, 51), and an IFA assay has been used for the presumptive diagnosis of cryptosporidiosis in two clusters of cases (86, 167). Specific anti-*Cryptosporidium* IgG, IgM, or both were also detected, by an enzyme-linked immunosorbent assay (ELISA), in the sera of 95% of patients with cryptosporidiosis at the time of medical presentation and in 100% within 2 weeks of presentation (335). With ELISA, one study in children showed a marked cryptosporidial antibody response (IgA, IgG, and IgM) in serum (176). *Cryptosporidium*-specific antibodies were also identified by ELISA in sera from 15 of 16 Thai children with cryptosporidiosis and in sera from 17 of 19 children from the same orphanage (147). In contrast to the orphans in Thailand, only 1 of 18 sera from toddlers in day-care centers in Denver, Colo., had antibodies specific to *Cryptosporidium* sp. (147). Several serologic surveys have reported that >50% of persons with no known infection may have anti-*Cryptosporidium* IgG, suggesting recent exposure to the parasite (167, 327, 332). One 2-year serologic survey of 56 United States Peace Corps volunteers in Africa reported an approximately 14% seroconversion rate each year (333). The limited seroprevalence data now available suggest that *Cryptosporidium* infections, many perhaps asymptomatic, are more common than the infection rates reported in surveys based on detection of fecal oocysts. Additional evaluations are needed to confirm the utility of these serologic procedures for diagnosing and monitoring infections, for determining the prevalence of prior exposure in selected study populations, and to determine whether there is any correlation between the presence of *Cryptosporidium*-specific serum antibodies and resistance to reinfection.

Atypical Oocysts

Beginning in 1987, researchers at the University of Liverpool reported small spherical bodies in the feces of children with diarrhea and considered these bodies to be "atypical oocysts of *Cryptosporidium*" (25). The possible existence of atypical oocysts that do not stain with the commonly used acid-fast techniques and that are associated with diarrheal illness is of concern to clinical microbiologists and to others faced with the diagnosis and treatment of gastrointestinal disease.

The small spherical bodies were considered by Baxby and Blundell (23) to be atypical *Cryptosporidium* oocysts because of their staining properties, osmotic instability, reactivity with a monoclonal antibody, reactivity with immunoglobulins in the sera of patients with confirmed cryptosporidiosis, and fine structure. Atypical oocysts did not retain Ziehl-Neelsen/Kinyoun, auramine-phenol, or safranin-methylene blue stains, all of which reacted strongly with *C. parvum* oocysts. The pink refractive appearance of atypical oocysts in Sheather's solution (sucrose-phenol) was similar to that of *C. parvum* oocysts; however, the atypical oocysts collapsed and were no longer detectable after 6 to 10 min. Atypical oocysts exhibited little or no reactivity with a monoclonal antibody used in a commercially available, direct immunofluorescence test for the detection of *C. parvum* oocysts. When sera from patients (number not reported) with confirmed *C. parvum* infections were used in an indirect IFA assay, both *C. parvum* oocysts and the atypical oocysts exhibited similar fluorescent reactivity. It is our belief that the transmission electron micrographs presented by Baxby and Blundell (23) reveal little or no similarities in the ultrastructure of atypical oocysts and oocysts of *C. parvum* (81, 256). The round bodies considered by the authors to be cross sections of sporozoites in the atypical oocysts are more likely profiles of mitochondria within the cytoplasm of an unidentified protozoan or fungus. It is possible that the atypical oocysts described by Baxby and colleagues (23, 25) represent a life cycle form of an unrecognized organism that is responsible for diarrheal illness in some children; however, we do not believe that they are a life cycle form of any species of *Cryptosporidium*.

TREATMENT

Chemotherapy

The lack of an effective treatment of cryptosporidiosis in previously healthy, immunocompetent persons has not been of major concern to the biomedical community since in such patients the duration of diarrhea is almost always <20 days and clinical symptoms and oocyst shedding generally resolve spontaneously. However, reports (39, 147, 259) demonstrating an association between cryptosporidiosis and severe malnutrition in children may warrant a change in perception. If a safe and effective therapy were available, most clinicians would probably treat the severe, short-term diarrheal illness

that often develops in immunocompetent persons following oral exposure to oocysts. Supportive care with oral or intravenous hydration, often with parenteral nutrition, provides a clear benefit to most immunocompetent patients (101).

Because immunocompromised persons often develop a prolonged life-threatening infection following exposure to the parasite, an effective therapy is desperately needed to treat cryptosporidiosis in this patient population. To date, treatment of cryptosporidiosis in immune deficient persons has been unsuccessful in most cases. No controlled studies have been published, and all therapeutic information is based on isolated reports. The list of unsuccessful attempts to treat cryptosporidiosis in immunocompromised persons is growing rapidly and includes the use of more than 90 different therapeutic and preventive modalities (73, 75, 101, 113, 126, 157, 294). Anecdotal success has been reported with diloxamide furoate (55), furazolidone (55), quinine plus clindamycin (56), and interleukin-2 (159). One paper reporting anecdotal success with amprolium for treatment of human coccidiosis (339) has been cited by others as evidence of the effectiveness of this drug against cryptosporidiosis; however, the patients treated were suffering from isosporiasis (*Isospora belli* infection).

Inconclusive results have been reported for oral treatment with spiramycin, a macrolide antibiotic related to erythromycin (64, 97, 120, 248, 253, 272, 350, 354). An evaluation of 37 patients with cryptosporidial diarrhea who were treated with spiramycin prompted investigators to conclude that 28 had a favorable response in the reduction of the number of daily bowel movements to <50% of that prior to treatment and that 12 of the 28 stopped shedding oocysts (228a). Unfortunately, details regarding other concurrent infections and specific clinical data were lacking for these patients. In a young, healthy, immunocompetent male with prolonged symptomatic cryptosporidiosis, clinical improvement was seen after a course of spiramycin. Family members who also suffered chronic undiagnosed diarrhea became asymptomatic after spiramycin therapy (97). In one report, 5 of 10 immune deficient patients with cryptosporidiosis had complete resolution and 4 had symptomatic improvement after 1 week of spiramycin therapy (253). In an AIDS patient with cryptosporidiosis involving the pancreas, biliary tree, bowel, and respiratory tract, the diarrhea resolved after spiramycin treatment for 4 weeks, but he continued to shed oocysts (120). In another report, five AIDS patients with intestinal cryptosporidiosis had reductions in stool volumes, but side effects limited the length of therapy. Oocysts were still found in stool samples obtained following treatment (64). From the data reported at the time of this writing, it appears that spiramycin may help control the diarrhea in some patients treated for cryptosporidiosis during the early stage of AIDS, but the drug does not appear to have a marked effect on the course of clinical cryptosporidiosis in patients who have progressed to the later stages of AIDS (295). In a study of cryptosporidial diarrhea in infants, 21 of 39 patients received spiramycin and 18 received a placebo, with no difference in outcome reported between the two groups (350).

Other studies are evaluating alpha-difluoromethylornithine, which is active against other protozoa such as *Eimeria tenella*, *P. carinii*, and the African trypanosomes (118, 208, 225). However, side effects such as bone marrow suppression and gastrointestinal irritation have limited its use (295).

In the absence of an effective treatment for cryptosporidiosis, supportive therapy appears to be the only intervention available to most clinicians. Oral and parenteral rehydration therapy is often required by both immunodeficient and immunocompetent persons, especially young children, with severe cryptosporidial diarrhea. Parenteral nutrition may also help sustain the nutritional status of some patients with persistent cryptosporidiosis. Antidiarrheal compounds may also be of some value in controlling fluid loss. Several antidiarrheal compounds have been reported to provide symptomatic improvement. Subcutaneous administration of a somatostatin analog, SMS-201-995, significantly reduced the number of daily bowel movements and the stool volume from an AIDS patient who had cryptosporidial diarrhea for at least 10 months (65). Diphenolylate treatment was reported to be effective in reducing the number of daily bowel movements until adverse side effects necessitated discontinuation (64). Seven patients given long-acting morphine sulfate had at least a 50% reduction in stool volume, and stools became formed in six patients (64).

A number of drugs have also been evaluated for anticryptosporidial activity, using animal models. None of the 15 anticoccidial compounds tested prophylactically in a suckling mouse model prevented infection, even at high doses (10). However, amprolium, arprinocid, dinitolmide, salinomycin, and sulfaquinoxaline reduced the number of oocysts produced compared with that of the untreated controls. The most efficacious of these drugs, arprinocid, reduced but did not completely inhibit development of *C. parvum* in neonatal hamsters (163), was ineffective in mice when administered therapeutically (328), and failed to control cryptosporidiosis in lambs (10). Lasalocid, a polyether ionophore antibiotic, was reported to be effective for prophylaxis against experimental *C. parvum* infections in calves but was thought to be toxic at the levels administered (226). More recently, data were presented suggesting that sulfadimethoxine has activity against *C. parvum* in an immunosuppressed rat model (259). Additional studies on sulfa compounds may be warranted.

Immunologic Intervention

Since the immune status of the host appears to be a major factor determining the severity and duration of infection following oral exposure to *C. parvum* oocysts (77) and since an effective therapy is not available (75, 113), immunologic intervention may be one approach to the control of cryptosporidiosis (14, 46, 51, 98–101, 188, 206, 212, 214, 221, 227, 275, 306, 329, 332, 334). Discontinuation of immunosuppressive chemotherapy, allowing restoration of immune function, has resulted in complete resolution of intestinal cryptosporidiosis in several patients (221, 306). One other approach directed toward restoration of immune function has also been reported to be of some value in treating cryptosporidiosis in AIDS patients. In one study of 14 patients with AIDS and a history of cryptosporidial diarrhea for at least 1 month, 7 were treated with a specific bovine dialyzable leukocyte extract (immune DLE) prepared from lymph node cells from calves immunized with *C. parvum*, and 7 were treated with a nonspecific (nonimmune) DLE prepared from nonimmunized calves (212). Six of seven patients receiving weekly oral doses of immune DLE gained weight and had decreased bowel movement frequencies, with eradication of oocysts from stool in five patients. In contrast, only one of seven patients given nonimmune DLE had a decrease in bowel movement frequency, only two gained weight, and four remained stool positive for oocysts. After subsequent treatment of five of these seven patients with immune DLE, four had decreased bowel movement frequency and weight gain, and oocysts were eradicated

from stool of two patients. This study suggests that oral administration of an uncharacterized dialyzable extract prepared from lymph node cells obtained from calves immune to *C. parvum* (immune DLE) may produce sustained symptomatic improvement in patients with AIDS and cryptosporidiosis. Additional studies are needed to confirm the observations noted in these patients and to determine whether such treatment results in augmentation of cellular immunity to *C. parvum*.

Although *Cryptosporidium*-specific IgA, IgM, and IgG responses are detectable in sera by ELISA and IFA procedures (46, 51, 176, 215, 327, 332, 334), the role these antibodies play in protective immunity is questionable. Because the parasite appears to be confined to the mucosal surface and because numerous studies have failed to demonstrate a protective role for serum antibodies against closely related species of coccidia (270), it is more probable that secretory antibodies coupled with cell-mediated immune mechanisms are responsible for the clearance of parasites from the infected mucosa and for rendering the immunocompetent host resistant to reinfection. The role of secretory antibodies as mediators of protective immunity to *Cryptosporidium* infections merits further investigation. The presence of *Cryptosporidium*-specific secretory antibodies has been reported in stools of Philippine children (176) and in feces obtained from experimentally infected lambs (137). Antibody neutralization-sensitive epitopes on the surface of *C. parvum* sporozoites have been demonstrated, and several laboratories are investigating the potential immunotherapeutic utility of hyperimmune bovine colostrum (see below).

Mata et al. (206) reported that breast-fed infants in Costa Rica had a significantly lower incidence of cryptosporidiosis than did age-matched babies in the same study populations who were fed artificial diets, and they proposed that lactogenic immunity may play an important role in controlling *C. parvum* infections. Similar studies in Ecuador, Guatemala, Haiti, and Liberia also revealed that breast-fed infants rarely had cryptosporidiosis (101). These studies did not provide data that make it possible to determine whether biologically active factors in milk or reduced exposure to contaminated food and water was responsible for the lower prevalence of cryptosporidiosis in breast-fed children.

The concept of passive lacteal immunity was subsequently tested in several studies to determine whether antibodies in milk or colostrum can prevent or abrogate intestinal infections with *C. parvum*. Colostrum or milk from dairy cows exposed naturally to the parasite does not appear to protect calves or humans from *C. parvum* infection; however, colostrum from hyperimmunized cows may provide some protection. We routinely administered oocysts of *C. parvum* to 1-day-old calves along with the first of 3 liters of colostrum. The subsequent course of infection was not affected by the presence of high titers of *Cryptosporidium*-specific antibody in the colostrum; the parasites excyst, invade, and then replicate within the intestinal mucosa when high levels of colostrum antibody are present in the gut lumen. That most calves will experience cryptosporidiosis while they are nursing from cows, most of which have colostrum antibodies to *C. parvum*, also supports the concept that natural exposure to the parasite does not result in significant lactogenic immunity. Oral administration of colostrum from a nonimmunized, naturally exposed dairy cow, which contained antibodies to *C. parvum*, did not alter the course of infection in an AIDS patient with cryptosporidiosis (275). A similar lack of lactogenic immunity was also reported in infant mice whose dams were immunized by oral inoculation of *C. parvum* oocysts (14, 227).

In contrast to the above reports, several studies indicate that colostrum obtained from cows hyperimmunized with oocyst/sporozoite antigens of *C. parvum* may protect humans and mice from cryptosporidiosis. Tzipori et al. (329) reported that three immune deficient patients recovered from cryptosporidiosis within 3 to 5 days after initiation of oral administration of hyperimmune cow colostrum produced by immunizing pregnant cows with concentrated *C. parvum* oocyst/sporozoite antigens. Two of the patients had subclinical infections following treatment, and the other remained free of infection for several months after the treatment was stopped. Fayer et al. (100) demonstrated that hyperimmune bovine colostrum, obtained from cows immunized with purified *C. parvum* oocysts, neutralized sporozoites and protected mice from oocyst challenge. Significantly fewer stages of *C. parvum* were found in suckling mice that were given whey from hyperimmune colostrum (undiluted or diluted 1:20 or 1:50) before and after oocyst inoculation than in mice given whey from control colostrum. Significantly fewer stages were also found in mice following intrarectal inoculation of sporozoites incubated in hyperimmune whey (diluted 1:20 or 1:50) than in mice receiving sporozoites incubated in similar dilutions of control whey. Fayer et al. (98) also reported that this hyperimmune colostrum provided prophylaxis against cryptosporidiosis in calves. Calves given hyperimmune colostrum 2 days after oral inoculation of *C. parvum* oocysts had less diarrhea and shed oocysts for a shorter period of time than did calves given nonhyperimmune colostrum 2 days after oocyst inoculation. Although these studies indicate that some component of hyperimmune bovine colostrum may exhibit anti-*Cryptosporidium* activity, additional studies are needed to define further the role of lactogenic immunity in preventing and treating cryptosporidiosis and to isolate and characterize the components of hyperimmune colostrum or milk that are responsible for the reported protective effects. A more recent study has shown that the immunoglobulin fraction purified from hyperimmune bovine colostrum has an immunotherapeutic effect on cryptosporidiosis in neonatal mice (99). Western blot (immunoblot) analysis revealed that hyperimmune bovine colostrum recognizes more than 40 *C. parvum* oocyst/sporozoite antigens separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (314). Some of the strongly recognized sporozoite antigens within the range of 15 to 25 kDa are known to be highly immunogenic, and monoclonal antibodies to some of these have been effective in reducing the severity of *C. parvum* infections in experimentally infected mice (14, 264, 265).

HOST RESISTANCE AND ACQUIRED IMMUNITY

Our limited understanding of host resistance to *Cryptosporidium* infections can be discussed within the context of data suggesting that age resistance occurs in some host species but not in others and that acquired immunity is the usual outcome of a primary infection. Age at the time of exposure appears to have different effects, depending on host species, on the susceptibility and/or the course of infection following exposure to oocysts of *Cryptosporidium* spp. Available data relating to age resistance and/or acquired immunity are somewhat confusing and can best be discussed within the context of the different host species.

Humans

In humans, age and immune status at the time of primary exposure to *C. parvum* do not appear to be primary factors influencing susceptibility to infection: symptomatic intestinal and respiratory cryptosporidiosis has been reported in both immunocompetent and immunodeficient children and adults (8, 73, 101, 322). However, host immune status does have a marked impact on the length and severity of human cryptosporidiosis. Immunocompetent persons usually develop a short-term (<2 weeks), self-limited, diarrheal illness following oral exposure to *C. parvum* oocysts, whereas most immunocompromised persons initially develop a similar illness that becomes progressively worse with time, resulting in a prolonged, life-threatening, choleralike illness. Such prolonged life-threatening infections have been reported in patients undergoing immunosuppressive chemotherapy with drugs that affect both T- and B-lymphocyte function (8, 71, 73, 101, 322), in at least one person with hypogammaglobulinemia with reported normal T-lymphocyte function (82), and in patients with AIDS (8, 73, 101, 322). These observations suggest that the marked difference in outcome between the immune deficient and the immunocompetent person exposed to *C. parvum* can probably be best explained by the development of an acquired immune response of sufficient magnitude to clear the parasite from the intestinal mucosa. This concept is also supported by reports of persons who rapidly cleared *C. parvum* infections when their immune function was restored following discontinuation of immunosuppressive chemotherapy (221). In a recent study, the authors used ELISA to measure *Cryptosporidium*-specific IgA, IgG, and IgM antibody levels in serum, stool, and duodenal fluid of 15 children. They concluded that the immune response to cryptosporidiosis in their subjects was probably an antibody-dependent, cell-mediated, cytotoxic effect of unknown mechanism (176).

Nonhuman Primates

Reports of intestinal, hepatopancreatic, and pulmonary cryptosporidiosis in immunocompetent, infant, rhesus, and other macaques have been attributed to an organism with oocysts indistinguishable from those of *C. parvum* (59, 171, 349). Miller et al. (220) reported 81 cases of acute cryptosporidiosis among 152 infant primates, predominantly *Macaca nemestrina*, housed in the nursery unit of the Washington Regional Primate Research Center. All but one of the animals had symptoms, predominantly diarrhea and dehydration, similar to those seen in immunocompetent human infants, i.e., intestinal cryptosporidiosis followed by spontaneous resolution. The authors also reported a striking absence of secondary or chronic cases of cryptosporidiosis in the population and found oocysts in the feces of only 1 of 180 adult and juvenile animals housed in other rooms within the primate colony. These observations suggest the development of acquired immunity to *C. parvum*; however, the aspect of age resistance in these animals could not be ruled out.

There are also reports of cryptosporidiosis in immunodeficient infant, juvenile, and adult macaques infected with the simian immunodeficiency virus (134, 164, 165, 178–180, 209, 240). These reports suggest that nonhuman primates are similar to humans with respect to susceptibility to *C. parvum* infection. It appears that macaques of all ages are susceptible to developing clinical cryptosporidiosis, especially when they are immunodeficient. The higher prevalence of *C. parvum* infections in infants may, therefore, be attributed to the development of acquired immunity that renders juveniles and adults resistant to oral challenge rather than to age resistance. Serologic surveys for the presence of *Cryptosporidium*-specific antibodies in animals within large primate centers may help resolve this question.

Cattle

Much of the published literature presents bovine cryptosporidiosis as a disease of neonates and not older animals, leaving the impression that age resistance may be a major factor forcing the parasite to "carve out" a niche within the youngest animals of the herd. This interpretation may be oversimplified since it appears that most calves are exposed to *C. parvum* during the first month of life (73) and that such exposure results in marked resistance to reinfection. Thus, a more careful consideration of available data, including reports of naturally acquired infections in adult cattle (242) and mild experimental infections in previously unexposed animals over 3 months of age (72), suggests that both acquired immunity and host age may be important in determining susceptibility to and severity of infection following exposure to *C. parvum* oocysts. It is likely that the individual contribution of these two factors to the resistance observed in the adult bovine will be difficult to resolve because of the complexity and expense involved when cattle are used as experimental animals.

Even though the development of acquired immunity seems to be the best explanation of the difference in the course of infection in immunocompetent and immune deficient humans, nonhuman primates, and cattle, our understanding of the immune mechanisms and parasite antigens involved is quite limited. Perhaps the major factor responsible for our present limited knowledge of acquired immunity to *Cryptosporidium* spp. is the lack of an adult, immunocompetent, rodent model. An immunocompetent rodent (preferably mouse) model is needed to elucidate the mechanisms involved in development of acquired immunity to this small coccidian parasite.

Laboratory Rodents

Mice. The most widely used laboratory animal model for cryptosporidiosis is the suckling mouse. Suckling mice rather than adults are used routinely because of an apparent age-related resistance that occurs in most laboratory rodents. Early experience with *C. parvum* demonstrated that virtually all conventional (as opposed to germfree or immune deficient strains) suckling rodents, i.e., mice, rats, cotton rats, hamsters, and guinea pigs, develop heavy intestinal infections after oral inoculation of 10^3 or more oocysts of human and calf isolates (76, 81, 82). However, previously unexposed rodents more than 3 to 4 weeks of age are difficult to infect; the parasite often cannot be found even after inoculation of more than 2×10^6 oocysts, and if found, they are observed in very small numbers within the intestinal mucosa (124, 132, 257, 283). Several studies have been conducted in an attempt to address the most obvious causes of this marked difference in susceptibility between neonate and adult mice, i.e., differences in gut physiology and microflora or differences in immune status.

Our understanding about differences in the gut microflora and physiology of adult and neonate laboratory rodents which affect the ability of *C. parvum* to colonize and establish infections is limited. We are aware of a single

published study that has addressed this aspect. Harp et al. (124) used two strains of adult and infant mice in an attempt to determine the effect of the adult gut microflora on establishment of *C. parvum* infections. In one experiment, using adult mice of the same strain (CD1), 7 of 9 antibiotic-treated germfree mice developed light to moderate infections following oral inoculation of *C. parvum* oocysts, whereas only 4 of 12 antibiotic-treated conventional mice developed light infections. Since bacteria could not be cultured from the intestines of mice in either group, the authors argue that the increased susceptibility of the germfree mice was not due to the absence of flora that may block intestinal colonization of *C. parvum* by competing for receptor sites on host cells, producing anticryptosporidial agents, or stimulating gut motility. However, the authors did suggest that antigenic stimulation provided by the adult gut flora in the conventional mice could be responsible for activating components of the immune system mediating resistance against *C. parvum*. In their second experiment, none of 15 antibiotic-treated or 14 nontreated adult BALB/c mice developed infections following oocyst challenge; however, all 7 infant mice developed heavy infections. Although results of the second experiment were consistent with the authors' hypothesis that activation of the immune system by previous association with adult intestinal flora contributes to *C. parvum* resistance, additional studies are needed to confirm or refute this concept.

Our early attempts to establish infections in adult mice by the use of immunosuppressive chemotherapy were disappointing. Adult Swiss-Webster mice administered cyclophosphamide did not develop more consistent or heavier infections than did nonimmunosuppressed mice following oral inoculation of *C. parvum* oocysts; only light infections lasting a few days could be demonstrated (by oocyst shedding and/or microscopic examination of the intestinal mucosa) in about half of the mice in each group (257). Sherwood et al. (283) also reported that cyclophosphamide administration did not alter the susceptibility of adult mice to *C. parvum*. Such results are difficult to interpret because immune parameters were not monitored in these mice before, during, or after inoculation with the parasite. It is possible that, because of insufficient T-lymphocyte depletion, the overall immune status of the mice in both studies was not significantly altered. Additional studies are needed to define more clearly the effect of immunosuppressive chemotherapy on the development and intensity of *C. parvum* infection in adult mice.

Several studies have focused on the use of T-cell-deficient nude (*nu/nu*) mice as potential laboratory models for cryptosporidiosis. One study compared oocyst-induced *C. parvum* infections in infants and adult *nu/nu* mice (132). Heavy intestinal infections in all infants and light infections in about half of the adults were observed when animals were monitored for approximately 2 weeks following oral inoculation of *C. parvum* oocysts into athymic nude mice and their immunocompetent heterozygous (*nu/+*) littermates. In this study, infections in adult mice were monitored for 2 weeks only. When the course of experimentally induced infections in 6-day-old *nu/nu* mice and their *nu/+* immunocompetent littermates (132) was monitored over a longer period of time, the T-cell-deficient nude mice developed diarrhea and shed oocysts in their feces until they died or until the experiment was terminated at 56 days; however, the heterozygous littermates did not develop diarrhea and stopped shedding oocysts 21 to 30 days after inoculation. These early studies suggested that host immune status at the time of parasite inoculation may not be the predominant factor determining susceptibility to *C. parvum* infection in the mouse gut; however, functional T lymphocytes are important for the clearance of *C. parvum* from the mammalian intestine.

More recent studies suggest that this may be an oversimplistic view of the many complex events that may affect parasite colonization and subsequent development in the gut. Ungar et al. (331) reported that severe intestinal and hepatobiliary infections with *C. parvum* were produced in adult BALB/c *nu/nu* mice; however, clinical signs of cryptosporidiosis and large numbers of oocysts in the feces did not appear until 3 weeks after inoculation. Thus, it appears that adult BALB/c *nu/nu* mice are susceptible to *C. parvum* infection but that it takes at least 3 weeks for large numbers of parasites to develop within enterocytes of the exposed mucosal site. In contrast, heavy infections develop in neonates (immunocompetent or *nu/nu*) within 3 to 5 days after inoculation. The authors also reported that similar persistent infections could be established in immunocompetent mice when they were depleted of CD4 cells by treatment with specific monoclonal antibodies and inoculated with *C. parvum* as neonates.

From the data reviewed above, it appears that oral inoculation of *C. parvum* into adult laboratory mice may result in the establishment of a small number of parasites in the intestinal mucosa. If the inoculated mice are immunocompetent, the infection is cleared, often before the parasite population becomes large enough to detect the infection. If, however, the inoculated mice are rendered immunodeficient by depletion of T-helper lymphocytes, the small number of parasites colonizing the intestine of some mice can increase over the subsequent 3 to 4 weeks and become sufficiently large to produce disease.

In contrast to laboratory mice, adult wild mice have been reported (166) to be easily infected with *C. parvum* and to develop heavy infections within 1 week of oral inoculation of oocysts. These observations suggest that genetically based as well as age- and immunity-related factors may be responsible for determining susceptibility or resistance to establishment of heavy *C. parvum* infections in adult mice. It is possible that the laboratory mouse of today differs genetically in susceptibility to *C. parvum* from those used by Tyzzer during the early 1900s in his detailed study (320) of the life cycle of this parasite. Additional studies are needed to determine the age, genetic, and immunologic factors responsible for resistance or susceptibility of adult mice to *C. parvum* infections.

Rats. Several recent studies suggest that host immune status may play a more important role than age in the susceptibility of rats to *C. parvum* infection. Rheg et al. (258) described an adult cyclophosphamide-treated rat model in which 50 mg of drug per kg per day in the drinking water for 14 days prior to oral inoculation of *C. parvum* oocysts resulted in heavy, persistent, intestinal infections as long as the animals were maintained on immunosuppression. Brasseur et al. (42) and, more recently, Rheg et al. (258) reported that a regimen of immunosuppression similar to those used to induce *P. carinii* infections rendered adult Sprague-Dawley rats susceptible to persistent intestinal cryptosporidiosis following oral inoculation of *C. parvum* oocysts. Cessation of corticosteroid immunosuppression resulted in rapid clearance of the parasites (as determined by oocysts in feces); however, reinitiation of immunosuppression at any time during a 10-week convalescent period caused at least some of the animals to begin shedding oocysts again. The authors' proposal of a 10-week latent period following withdrawal of immunosuppression should be viewed with cau-

tion since the rats may have been re-exposed to oocysts, which can survive for a long period of time in a moist environment.

Guinea pigs. At present, there appears to be one exception to the concept that immunocompetent, adult, laboratory rodents do not develop heavy intestinal infections following oral inoculation of *Cryptosporidium* oocysts. Angus et al. (11) described *Cryptosporidium* infections in immunocompetent guinea pigs of all ages. Chrisp et al. (57) studied what appears to be the same organism and reported that 6-week-old guinea pigs developed moderate to heavy infections that were cleared within 4 weeks. Once the infection was cleared, the animals were resistant to reinfection and antibodies specific to oocysts were detected by an immunoperoxidase technique. Our studies with the same isolate confirm these data. Oral inoculation of oocysts resulted in large numbers of parasites within the microvillous region of enterocytes throughout the small intestine and a few parasites in the cecum and colon. We have also shown that guinea pigs shed oocysts in their feces for approximately 3 weeks after oral inoculation and that, once the infection is cleared, the animals are resistant to oral challenge with the same isolate. Antibodies specific to *Cryptosporidium* sp. appear in the sera of guinea pigs during the time they are clearing a primary infection and remain at detectable levels for several weeks (353).

ANTIGENS

From the discussions above, it is apparent that host immune status is a major determinant of the severity of cryptosporidiosis following exposure to oocysts and that acquired immunity is probably responsible for the clearance of an infection and for resistance to subsequent challenge. Each of these features has led to a search for antigens that may be important for the induction and/or expression of acquired immunity.

Potential Sporozoite and Oocyst Antigens

The development of techniques to purify oocysts of *Cryptosporidium* spp. and to separate sporozoites from intact oocysts and oocyst walls (76) has allowed several researchers to use electrophoresis and immunoblotting for general molecular weight determinations of proteins and glycoproteins of these life cycle forms (111, 190–192, 215, 264, 314–316). These studies have identified more than 50 bands in electrophoretic profiles of sporozoites or oocysts or both. Tilley and Upton (315) used SDS-PAGE, immunoblotting, lectin binding, and iodine-125 surface labeling to characterize the proteins and glycoproteins of purified sporozoites and oocysts of *C. parvum*, the mammalian pathogen, and *C. baileyi*, a species that infects poultry. Silver-stained profiles of freeze-thawed oocysts revealed more than 50 bands, while profiles of sporozoites exhibited more than 40 bands. Surface iodination of sporozoites revealed approximately 20 surface proteins; the most heavily labeled ones formed bands corresponding to 18 to 20, 37 to 39, 48, 73 to 76, and 102 to 105 kDa. Following electrophoresis and Western blotting, 4 of 12 different ^{125}I-labeled lectin probes collectively bound to at least 19 bands, indicating that numerous sporozoite proteins of *C. parvum* are glycosylated.

In addition, studies in one of our laboratories (W.L.C.) have been initiated to develop an "antigenic library" for both *C. parvum* and *C. baileyi*, species readily recognized by morphology of life cycle stages and host specificity. Hyperimmune serum produced by immunization of rabbits with purified *C. parvum* oocyst walls and/or sporozoites recognize more than 75 electrophoretically distinct sporozoite and oocyst antigens of the same species and also exhibited remarkable cross-reactivity with sporozoite antigens of *C. baileyi* (84) (Fig. 9). This antigenic cross-reactivity also provides an opportunity to use *C. baileyi* oocysts as an antigen source for ELISA and IFA assay designed to detect *C. parvum* antibodies in various hosts, including calves and humans (74).

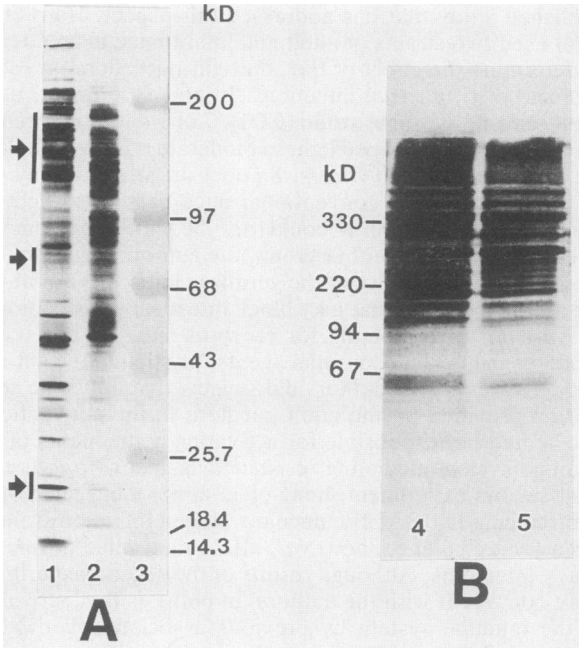

FIG. 9. Western blot (enzyme immunotransfer blot) of oocyst/sporozoite antigens of two species of *Cryptosporidium*, *C. parvum* and *C. baileyi*. Oocysts of *C. parvum* were purified from the feces of experimentally infected calves. Oocysts of *C. baileyi* were purified from the allantoic fluid of experimentally infected chicken embryos. Oocysts were placed in 10 mM Tris buffer (pH 8.0) with 2 mM phenylmethylsulfonyl fluoride and then ruptured by three freeze-thaw cycles (liquid nitrogen, +40°C). Following centrifugation (15,000 × *g*, 30 min), the protein concentration of the supernatent solution (soluble antigen) was determined (64). The soluble antigen and the pellet (oocyst wall and membrane-enriched antigen) were placed in sample buffer containing SDS and 2-mercaptoethanol and boiled for 5 min. Lanes of 5 to 15% gradient SDS-PAGE gels were loaded with 20 ml of the soluble or oocyst wall-membrane-enriched antigen (approximately 500 mg of protein/ml), electrophoresed, and transblotted onto nitrocellulose. The nitrocellulose sheets were probed with a 1:100 dilution of antisera from rabbits immunized with freeze-thaw-disrupted oocysts of *C. parvum*. The horseradish peroxidase-conjugated, affinity-purified, goat anti-rabbit IgG (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md) was diluted 1:1,000. Further details of the procedures used are outlined elsewhere (78). (A) Lanes: 1, soluble antigens of *C. parvum*; 2, soluble antigens of *C. baileyi*; 3. molecular weight markers. Arrows to the left indicate molecular weight regions in which antigens were reported (201, 298, 315) to react strongly with sera obtained from humans with confirmed exposure to *Cryptosporidium* sp. (B) SDS was included in the transfer (blotting) buffer to facilitate transfer of the high-molecular-weight antigens shown here. Lanes: 4, Soluble antigens of *C. parvum*; 5, soluble antigens of *C. baileyi*. Reproduced from reference 77 with permission of the publisher.

Antigens Recognized by Humans

Several studies have demonstrated that only a few of the proteins and glycoproteins identified as potential antigens in oocyst walls and sporozoites of *C. parvum* are recognized strongly by sera obtained from humans (215, 334) following their recovery from intestinal cryptosporidiosis (Fig. 9).

Ungar and Nash (334) reported that 37 of 40 serum samples from persons with cryptosporidiosis (24 AIDS and 16 non-AIDS patients) recognized, by Western blot analysis, a 23-kDa *C. parvum* sporozoite antigen that was separated by 5 to 15% SDS-PAGE under reducing conditions (5% mercaptoethanol). They also reported that 58 of 63 serum samples from IgM- or IgG-positive individuals, as determined by an ELISA, recognized the same antigen. In some of the sera, up to three additional bands between 125 and 175 kDa were also recognized strongly and a larger number of additional bands reacted weakly. These authors proposed that the 23-kDa antigen may be useful in serodiagnosis since sera from most infected persons, including AIDS patients, react strongly to this particular band. Mead et al. (215) reported that sera obtained from persons at various times (10 days to 1 year) after infection with *Cryptosporidium* sp. reacted strongly with a 20-kDa antigen separated on 10 to 20% gradient or 10% standard SDS-PAGE under reducing (mercaptoethanol) conditions. By using a specific monoclonal antibody (C6B6) and IFA and by biotinylation of the parasite, the authors provided evidence that the 20-kDa antigen was on the surface of *C. parvum* sporozoites. They concluded that the 20-kDa sporozoite surface antigen was probably the same as the 23-kDa antigen reported by Ungar and Nash (334), that the differences in reported molecular weights were due to differing gradient gel applications, and that the serum recognition of this antigen probably correlates with recent exposure to *C. parvum*. However, caution is advised when comparing Western blots. Results of recent studies (314–316) compared with others (191, 192, 215, 315, 334) suggest that at least two *C. parvum* sporozoite surface proteins are being confused. A 23-kDa molecule is weakly labeled by iodination (315), is highly immunogenic (191, 192, 215, 334), and may have several epitopes that cross-react with some higher-molecular-size species (192, 215). An 18- to 20-kDa protein, often referred to as P20, is intensely labeled by iodination (315) and appears to be less immunogenic than P23. Galactose or galactosamine residues have been detected on P20; however, P23 does not appear to be glycosylated (314, 315). Effects of preparation techniques on the degree of glycosylation or perhaps strain differences may explain the variation in size of P20 reported by different investigators (314, 315).

Unpublished studies from one of our laboratories (W.L.C.) suggest that immune sera from humans recognize more than the few antigens discussed above (215, 315, 334) and confirm that 20- to 23-kDa sporozoite antigens are not strongly recognized by sera from all individuals. Sera obtained from four animal handlers working with calves in Alabama (82) reacted with up to 20 (12 often react strongly) electrophoretically distinct *C. parvum* oocyst/sporozoite antigens, separated on a 5 to 15% gradient SDS-PAGE prior to Western blotting. Six of the antigens recognized by these sera are >200 kDa, four are between 100 and 200 kDa, and the remainder are between 25 and 97 kDa. Only one of the four serum samples from these persons who contracted cryptosporidiosis from exposure to infected calves recognized 20- to 23-kDa antigens. Using the same Western blot of SDS-PAGE-separated oocyst/sporozoite antigens, we found that sera obtained from 8 of 10 seropositive (by IFA) persons after a waterborne outbreak of cryptosporidiosis (86) were strongly reactive to a 20- to 23-kDa antigen. Some of these sera also recognized more than 18 electrophoretically distinct *C. parvum* antigens. Thus, it appears that, of the >75 electrophoretically distinct *C. parvum* oocyst/sporozoite antigens recognized by hyperimmune rabbit sera, <20 are usually recognized by immune sera from humans, and the recognition pattern observed on Western blots may vary among individuals. Such variation may be attributed to heterogeneity in humoral responses of infected individuals, possible differences in infecting isolates of *C. parvum*, and/or differences in antigen preparations and separation conditions.

Antigens Recognized by Mice

One study by Luft et al. (190) also suggests that sera from different hosts may recognize different oocyst/sporozoite antigens of *C. parvum*. Sera obtained from 6-week-old mice that were inoculated orally with 10^4 oocysts of *C. parvum* at 3 days and then weekly thereafter for 4 weeks recognized four antigens, with bands ranging from 72 to >100 kDa. These antigens also bound concanavalin A, suggesting that *C. parvum* antigens recognized by mice following intestinal exposure are carbohydrates alone or in association with lipids or proteins.

Studies involving the production of monoclonal antibodies to *C. parvum* (303) demonstrate that more than four high-molecular-weight sporozoite/oocyst antigens are recognized when parenteral immunization is used. One such monoclonal antibody (C6B6) recognizes a 20-kDa sporozoite surface protein, and another IgM monoclonal antibody presently marketed in an IFA diagnostic kit recognizes an antigenic epitope on the outer oocyst wall (109, 303). These monoclonal antibodies along with others developed against various life cycle stages of *C. parvum* should provide valuable tools, not only for diagnosis but also for identifying the biologic role of a number of different parasite antigens.

FUTURE DIRECTIONS

As both participants and observers during the past 8 years, we have found it interesting and exciting to monitor reactions within the biomedical community as the perception of *Cryptosporidium* sp. changed from that of a rare opportunistic pathogen to that of an important worldwide cause of diarrheal illness in humans and domesticated animals. During this short time period, the number of papers in the literature on *Cryptosporidium* spp. has gone from <30 to approximately 1,000. Despite the large number of recent papers and the large number of laboratories throughout the world devoting significant effort in *Cryptosporidium* research, our present understanding of this protozoan parasite is very limited.

Results of surveys (Table 2) using improved diagnostic techniques on patients with diarrheal illness or other gastrointestinal symptoms suggest that *Cryptosporidium* sp. is one of the three most common enteropathogens causing diarrheal illness worldwide (174a). However, additional large-scale studies in different geographic regions are needed to define more clearly the overall role of intestinal cryptosporidiosis as a cause of morbidity and mortality in immunocompetent and immune deficient persons. The prevalence and importance of extraintestinal manifestations, especially respiratory infections, of *Cryptosporidium* sp.

also merit further investigation. Improvements in diagnostic techniques and increased awareness in the biomedical community should result in a more clear definition of these issues.

Significant advancements in our understanding of several aspects of *Cryptosporidium* biology and cryptosporidiosis are to a great extent dependent on development of suitable in vitro cultivation and animal models. Suitable in vitro models are needed to investigate host cell-parasite interactions, to delineate metabolic pathways, to identify parasite-specific molecular targets for drug intervention, and to provide an adequate supply of organisms for immunology- and molecular biology-based investigations. More suitable laboratory animal models are needed to define the mechanisms of induction and expression of acquired immunity to *Cryptosporidium* spp. and the pathophysiologic mechanisms by which the parasite or its metabolites damage the host mucosa. Animal models will also be of value in studies to delineate the pathophysiology and host immune response to extraintestinal infections of *Cryptosporidium* spp. Participants in a recent National Institutes of Health-sponsored workshop (174a) identified the development of in vitro and animal models, ones that can be used to address those aspects of the basic biology of *Cryptosporidium* spp. that will lead to a more rational scientific approach to controlling cryptosporidiosis, as high-priority research efforts that should be supported.

Even in the absence of in vitro models that allow propagation and purification of large numbers of parasites, immunology- and molecular biology-based research programs are being established. Such programs are possible because of the development of techniques to purify large numbers of oocysts and sporozoites of *Cryptosporidium* spp. (76). Several laboratories are now developing monoclonal antibodies and are using them as reagents to probe the biology of *Cryptosporidium* spp. Use of these reagents in conjunction with the application of molecular biology techniques should result in many exciting and useful discoveries in the near future.

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